

From the Pediatric Graduate School  
Children's Hospital  
University of Helsinki  
Helsinki  
Finland

# **PERITONEAL DIALYSIS AND NEUROLOGICAL OUTCOME IN INFANTS AND SMALL CHILDREN**

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ACADEMIC DISSERTATION

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*To families with a child with  
a chronic kidney disease*

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# Abstract

**Background and aims.** Improved outcomes for children on peritoneal dialysis (PD) have been evident in recent years due to advances in medical care. However, the youngest patients, infants and small children, continue to demonstrate inferior growth, more frequent infections, and higher mortality compared to older children. Moreover, maintaining normal intravascular volume status, especially in young anuric patients, has proven difficult. There are relatively many children needing dialysis in infancy in Finland compared to other countries due to the high prevalence of congenital nephrotic syndrome of the Finnish type (NPHS1, CNF). While the youngest patients form the greatest challenges in PD treatment, the risk for neurological sequelae is higher than in older children. However, the neurodevelopment of infants and small children on PD is a rarely reported topic. This study was designed to treat and monitor these youngest PD patients with a strict protocol, to critically evaluate the results, and finally to improve metabolic balance, growth and development in infants and small children during PD.

**Methods.** A retrospective analysis of 23 children under two years of age at onset of PD (mean 0.4 years), treated between 1995 and 2000, was performed in the first study to obtain a control population for our prospective study. Data about diagnoses, dialysis period, medication, laboratory parameters, complications, and growth was collected from patient records.

For the second and third studies, 21 patients less than two years of age at the beginning of PD (mean 0.6 years) were enrolled in our prospective protocol between 2001 and 2005. In the second study, medication for uremia and nutrition were carefully adjusted during PD. Laboratory parameters and metabolic controls were regularly analyzed. To evaluate the intravascular volume status, blood pressure measurements, echocardiography, and analysis of N-terminal atrial natriuretic peptide (ANP-N) were performed. Growth before and during PD was analyzed and compared with midparental height. In the third study, the risk factors for development and the neurological development of these patients was determined. A neurologist, a physiotherapist and an occupational therapist evaluated all patients regularly during PD. The brain images taken before PD, during PD, and two years after renal transplantation (Tx) were surveyed. Hearing was tested during PD; in children at least three years old at the end of the study the hearing was tested also after renal Tx. Neuropsychological tests in children at least five years of age before the end of this study were collected and assessed.

In the fourth study, the data of six NPHS1 patients with a congruent neurological syndrome was analyzed. All these patients, born between 1984 and 2003, had a serious dyskinetic cerebral palsy-like syndrome with muscular dystonia and athetosis (MDA). They also had a hearing defect. The brain MRI showed increased signal intensity in T2-weighted images in the globus pallidus area and their neurological symptoms were detected before the age of one year. The analysis of mitochondrial DNA (mtDNA) in these NPHS1 patients with MDA was performed in order to find a possible genetic defect as an explanation for their neurological syndrome.

**Results.** Hospitalization time was clearly shorter in the prospective PD patient group (65 days per patient-year) compared to the retrospectively analyzed patient group (124 days per patient-year). Metabolic control was mainly good in both patient groups. However, the control of plasma intact parathyroid hormone level (iPTH) was very demanding. Furthermore, in the prospective patient group, although a mean weekly urea Kt/V of over 3.0 was achieved, the target of a mean creatinine clearance (Crcl) of over 65 L/week per 1.73 m<sup>2</sup> was very difficult, even impossible to achieve. It seems that a Crcl level of 40 L/week per 1.73 m<sup>2</sup> is acceptable in this age group. The peritonitis rate diminished, it was 1 per 17.8 patient-months in the prospective patient group versus 1 per 14.5 in retrospectively analyzed patients, although this difference was not statistically significant. Hypertension was common in retrospectively analyzed patients; 70% had antihypertensive medication while in the prospective patient group only 33% had antihypertensive medication at some point during PD. Thus, prospective patients received antihypertensive medication less frequently, but long-term hypertension was still seen in 43% during PD. Left ventricular hypertrophy decreased during the prospective study period. None of the patients in either group had pulmonary edema or dialysis-related seizures. Growth was good in most patients in both patient groups. Catch-up growth was documented in 64% of the retrospectively analyzed patients and in 57% of the prospective patients during dialysis. However, the prospective PD patients clearly lagged behind their midparental height at the end of PD. Mortality was 5% in the prospective PD patient group, and 9% in the retrospective PD patient group.

In the prospective PD patient group 11 patients (52%) had some risk factor for their neurodevelopment originating from the predialysis period. The neurological problems, detected before PD, did not worsen during PD and none of the patients developed new neurological complications during PD. Brain infarcts were detected in four patients (19%) and other ischemic lesions or periventricular leukomalacia (PVL) in three patients (14%). At the end of this study, 29% of the prospectively followed patients had a major impairment of their neurodevelopment and 43% only some minor impairment.

In the NPHS1-patients with MDA, neither mtDNA mutations nor external neurological complications could be found that could explain the symptoms. Thus, the reason for the neurological syndrome remains a mystery. Kernicterus was contemplated to be causative in the hypoproteinemic newborns but it could not be proven. Mortality was as high as 67% in this patient group during the whole follow-up period.

**Conclusions.** Our results for young PD patients were promising. Metabolic control was acceptable, growth was good, and mortality was low, however control of their calcium-phosphorus status proved demanding. Their peritonitis rate was about the same as for older children, whilst the high incidence of blood pressure proved problematic and several instruments were needed for examining and managing their intravascular volume status. Even if growth was good during PD, the children were significantly smaller compared to their midparental height. Although many patients were found to have neurological impairment at the end of our follow-up period, PD was a safe



treatment for end-stage renal failure (ESRF) in infants and small children whereby their neurodevelopment did not worsen.

*Key words:* peritoneal dialysis, congenital nephrotic syndrome of the Finnish type, NPHS1, neurodevelopment, risk factors, blood pressure, motor development, brain imaging, intravascular volume status, hypervolemia, neurological development, infants, adequacy of dialysis, metabolic control, growth, midparental height.

## List of original publications

This thesis is based on the following original publications, which will be referred to in the text by their Roman numerals:

- I Laakkonen H, Hölttä T, Lönnqvist T, Holmberg C, Rönholm K. Peritoneal dialysis in children under two years of age. *Nephrology Dialysis Transplantation* 2008 May; 23(5):1747-53.
- II Laakkonen H, Happonen J-M, Marttinen E, Paganus A, Hölttä T, Holmberg C, Rönholm K. Normal growth and intravascular volume status with good metabolic control during peritoneal dialysis in infancy. *Pediatric Nephrology* 2010 25:1529-1538.
- III Laakkonen H, Lönnqvist T, Valanne L, Karikoski J, Holmberg C, Rönholm K. Neurological development in 21 children on peritoneal dialysis in infancy. *Pediatric Nephrology* (in press).
- IV Laakkonen H, Lönnqvist T, Uusimaa J, Qvist E, Valanne L, Nuutinen M, Ala-Houhala M, Majamaa K, Jalanko H, Holmberg C. Muscular dystonia and athetosis in six patients with congenital nephrotic syndrome of the Finnish type (NPHS1). *Pediatric Nephrology* 2006 21:182-189.

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# Abbreviations

AIMS	Alberta Infant Motor Scale
ANP-N	N-terminal atrial natriuretic peptide
APD	Automated peritoneal dialysis
AV	Arteriovenous
BERA	Brainstem evoked response auditory
BIA	Bioelectrical impedance analysis
BOA	Behavioral observation audiometry
BP	Blood pressure
BSA	Body surface area
BUN	Blood urea nitrogen
CAPD	Continuous ambulatory peritoneal dialysis
CaCO <sub>3</sub>	Calcium carbonate
CCPD	Continuous cycling peritoneal dialysis
CKD	Chronic kidney disease
CNF	Congenital nephrotic syndrome of the Finnish type (NPHS1)
CNS	Central nervous system
CP	Cerebral palsy
Crcl	Creatinine clearance
CSF	Cerebrospinal fluid
CT	Computerized tomography
ECW	Extra-cellular water
EEG	Electroencephalogram
EF	Ejection fraction
EPO	Erythropoietin
ERA-EDTA	European Renal Association – European Dialysis and Transplantation Association
ESI	Exit-site infection
ESRF	End-stage renal failure
FAO	Food and Agriculture Organization of the United Nations
FGF23	Fibroblast growth factor 23
FRKD	Finnish Registry for Kidney Diseases
hcfSDS	Head circumference standard deviation score
HD	Hemodialysis
HDL	High-density lipoprotein
hSDS	Height standard deviation score
ICW	Intra-cellular water
IPP	Intraperitoneal pressure
iPTH	Intact parathyroid hormone
KDOQI	Kidney Disease Outcomes Quality Initiative
K	Dialyzer clearance of the measured molecule in Kt/V
Kt/V	Dialysis clearance measure

LDL	Low-density lipoprotein
LVEDD	Left ventricular end-diastolic dimension
LVESD	Left ventricular end-systolic dimension
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
LVMI	Left ventricular mass index
LVPWD	Left ventricular posterior wall thickness at end-diastole
MDA	Muscular dystonia and athetosis
MFED	Munich Functional Developmental Diagnostic test
MRI	Magnetic resonance imaging
MtDNA	Mitochondrial DNA
NAPRTCS	North American Pediatric Renal Transplant Cooperative Study
NEPSY	Developmental Neuropsychological Assessment
NKF	National Kidney Foundation
NNR	Nordic Nutrition Recommendation
NPHS1	Congenital nephrotic syndrome of the Finnish type (CNF)
<i>NPHS1</i>	Nephrin gene
NS	Nephrotic syndrome
(25-OH)D	Calcidiol
1,25(OH) <sub>2</sub> D	Calcitriol
pmarp	per million of age related population
PTH	Parathyroid hormone
PVL	Periventricular leukomalacia
PET	Peritoneal equilibration test
PD	Peritoneal dialysis
RDA	Recommended dietary allowance
rhGH	Recombinant human growth hormone
RI	Resistance index
RRF	Residual renal function
RRT	Renal replacement therapy
SeptD	Interventricular septal dimension at end-diastole
SGA	Small for gestational age
SNHL	Sensorineural hearing loss
t	Time in Kt/V
TBW	Total body water
TEOAE	Transient evoked otoacoustic emission
TG	Triglycerides
TI	Tunnel infection
TPD	Tidal peritoneal dialysis
Tx	Transplantation
UF	Ultrafiltration
ULN	Upper limit of normal
UNU	United Nations University
US	Ultrasound

V	Volume of distribution in Kt/V
vs.	Versus
WHO	The World Health Organization
WISC	Wechsler Intelligence Scale for Children
WPPSI	Wechsler Preschool and Primary Scale of Intelligence



# 1 Introduction

The first successful kidney transplantation (Tx) in an adult was performed in 1954 in Boston, USA (Merril et al. 1956) and ten years later, the first adult patient received a renal transplant in Finland (Flatmark 1989). Progress in surgical techniques, treatment of complications, as well as better immunosuppressive medication, have nowadays made kidney Tx a valid therapy for end-stage renal failure (ESRF).

The active treatment of childhood uremia was started in the late 1960's in Finland whereby some older pediatric patients even received kidney transplants in the adult unit. In 1971, the first small pediatric patient had a transplant operation at the Children's Hospital, Helsinki University Central Hospital, without success due to inadequate immunosuppression. The patient was hemodialyzed before transplantation. Thus, in the beginning only a few older children with ESRF were actively treated in Finland (Hölttä 2000c). Renal Tx in the youngest patients remained a controversial matter globally (Chantler 1979). However, much progress has subsequently been made and infants are today also included in Tx programs. Extra peritoneal placement of the kidney is used for all pediatric patients in Finland. Their body weight must be over 9 kg, which is usually reached at about one year of age, before an adult size kidney can be successfully transplanted. Infants with ESRF typically require dialysis before Tx as an intermediate phase, always aiming at renal Tx.

Continuous ambulatory peritoneal dialysis (CAPD) was commenced in children in the 1970's (Oreopoulos et al. 1979). In Finland, the first pediatric ESRF patients were treated with hemodialysis (HD) in units for adults. Peritoneal dialysis (PD) was introduced in pediatric patients in the early 1980's and for infants in the late 1980's (Hölttä 2000c). The latter group has been an especially challenging one because of the patients' small size and problems with growth and development. Today, most pediatric ESRF patients in Finland are treated with PD, especially the youngest ones.

Because of a high incidence of congenital nephrotic syndrome of the Finnish type (NPHS1, CNF), more Finnish infants need renal replacement therapy (RRT) when compared to other countries (van der Heijden et al. 2004). Infections, poor growth and higher mortality in infants compared to older children and adults have been the major problems encountered during their PD (Warady et al. 1997, Verrina et al. 2004, Bunchman 1995, Neu et al. 2002, Shroff et al. 2006). The PD period has also been shown to be the most crucial time for complications in small children with ESRF. For instance, hemodynamic crises may lead to serious complications (Qvist et al. 2002).

Neurological development of infants and small children on PD has not been extensively reported but developmental delay is documented in 11 to 84% during ESRF and PD (Polinsky et al. 1987, Geary, Haka-Ikse 1989, Honda et al. 1995, Warady et al. 1999), although improvement was seen after Tx in one study (Polinsky et al. 1987).

The present study was developed to critically evaluate the nutrition, medication, PD treatment, metabolic balance, intravascular volume status, growth, and neurological development, in order to improve the overall treatment of infants and small children during PD.





## 2 Review of the literature

### 2.1 End-stage renal failure in children

End-stage renal failure (ESRF) is the stage of renal disease where the patients' renal insufficiency has progressed so far that they cannot maintain their homeostasis and survive with their own renal function. At this point, renal replacement therapy (RRT), either dialysis or renal Tx, is needed (Harmon 1999).

#### 2.1.1 Incidence

The incidence of ESRF in children under 5 years of age has increased significantly with time in Europe; in 1980—1984 the incidence was 2.4 per million of age related population (pmarp), in 1985—1990, was 6.2 pmarp, and in 1995—2000, was 8 pmarp. However, in Finland the incidence of ESRF in children under 5 years was as high as 15.5 pmarp in 1995—2000 (van der Heijden et al. 2004) due to congenital nephrotic syndrome of the Finnish type (NPHS1, CNF). The incidence of NPHS1 is 1 in about 8200 live births in Finland (Huttunen 1976) which makes for a high proportion of small children in our ESRF population.

#### 2.1.2 Etiology

The most prevalent diseases leading to ESRF in young children in Finland (Finnish Registry for Kidney Diseases 2009), compared to 29 European countries (van der Heijden et al. 2004), are presented in Table 1. Malformations of the kidney and urinary tract, such as urethral valves, are not specified in the European Registry, but in Finland they constitute 9% and 10% of diagnoses in patients under 2 and under 5 years of age.

**Table 1.** Percentual proportions of renal diseases leading to ESRF in small children in Finland and Europe.

Registry	FRKD		ERA-EDTA
Age group	< 2 years	< 5 years	< 5 years
Hypoplasia/Dysplasia of kidneys	2 %	2 %	21 %
Hereditary nephropathy	72 %	63 %	19 %
Glomerulonephritis	2 %	4 %	13 %
Pyelonephritis	0 %	0 %	13 %
Hemolytic uremic syndrome	0 %	0 %	8 %
Cystic kidneys	7 %	11 %	6 %
Miscellaneous	17 %	20 %	12 %
Unknown	-	-	8 %

FRKD = Finnish Registry for Kidney Diseases 1990—2008

ERA-EDTA = European Renal Association - European Dialysis and Transplantation Association 1980—2000

### 2.1.2.1 Congenital nephrotic syndrome of the Finnish type

NPHS1 is the most common diagnosis leading to ESRF in Finnish infants (Qvist et al. 2002, Hölttä et al. 1997). It is an autosomal recessive hereditary nephropathy with mutations in the nephrin gene *NPHS1* (Holmberg et al. 2004). There are two main *NPHS1* mutations (Fin-major and Fin-minor) in Finland which both cause a complete absence of nephrin in the glomerulus and a severe defect in the slit diaphragm, the important filter in the glomerulus, between the podocytes (Kestilä et al. 1998, Patrakka et al. 2000, Putaala et al. 2001). This leads to severe proteinuria already in utero and is lethal without active treatment in early childhood. The patients are nowadays mostly nephrectomized during their first year of life to avoid the massive proteinuria and its consequences (Holmberg et al. 1995).

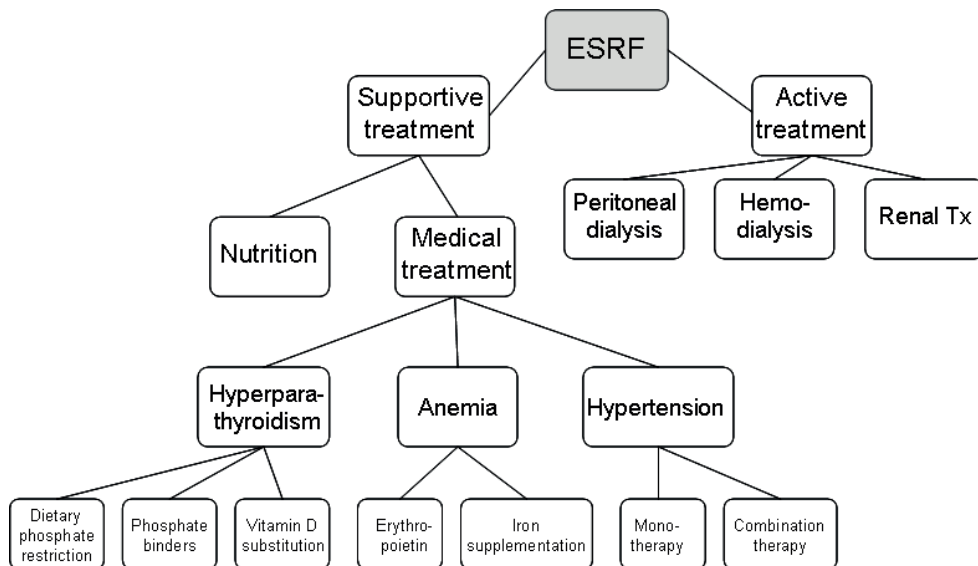
## 2.1.3 Consequences of end-stage renal failure

ESRF and nephrectomy have several consequences for the patients' health. The urinary excretion of phosphate decreases leading to hyperphosphatemia, active vitamin D diminishes, plasma calcium levels decrease, and all of these lead to increased parathyroid hormone (PTH) levels causing secondary hyperparathyroidism (see Fig. 3). These effects on bone cause high bone turnover and, with time, a bone growth disorder (Sanchez et al. 1999).

Fibroblast growth factor 23 (FGF23), a phosphatonin secreted by osteocytes and osteoblasts when serum phosphate increases, has been actively studied recently. FGF23 appears to be one of the key molecules in the regulation of phosphate homeostasis. It increases the secretion of phosphate into urine and thus decreases serum phosphate levels. FGF23 expression is also regulated by vitamin D; administration of 1,25(OH)<sub>2</sub>D, the active form of vitamin D, leads to increased levels of FGF23. Increasing FGF23 reduces 1 $\alpha$ -hydroxylase activity leading to a decrease in 1,25(OH)<sub>2</sub>D formation. This in turn increases PTH secretion (see Fig.3) (Silver, Naveh-Many 2010, Wesseling-Perry 2010, Yu et al. 2005, Isakova, Wolf 2010, Jüppner 2011).

Renal failure leads to metabolic acidosis due to reduced excretion of hydrogen ions. Acidosis induces hyperventilation, disturbs the normal metabolism of bones (causing growth retardation), and impairs the function of several hormones such as glucocorticoids, PTH, thyroid hormone and vitamin D (Mitch 2006). ESRF leads to high levels of waste products, such as urea nitrogen (BUN) and creatinine, cause many symptoms including nausea, fatigue, headache, and itching. Chronic uremia can also lead to more severe effects like pericarditis (Comty et al. 1971). Diminished production of erythropoietin in the kidneys leads to anemia (Wassner, Baum 1999). Anemia causes fatigue, dyspnea, deterioration of cognition, and stresses the heart among many other consequences. Edema and hypertension are also common findings in ESRF (Wassner, Baum 1999, Feld, Waz 1999, Haycock 1999).

## 2.2 Treatment of end-stage renal failure



**Figure 1** Treatment of end-stage renal failure (ESRF).

### 2.2.1 Supportive treatment

#### 2.2.1.1 Nutrition

Individualized nutritional counseling, frequent re-evaluations and modifications of the diet should be given to the PD patient and his/her family by a pediatric renal dietitian. The age, development, and the family situation should be taken into account when planning the nutrition of each child (KDOQI Work Group 2009).

#### Energy requirements

Children with ESRF should get 100% of the estimated energy requirement for their age, adapted to the body size and physical activity level (KDOQI Work Group 2009). In infants the daily energy intake should be roughly 80–90 kcal/kg. Additionally PD patients receive about 10 to 20 kcal/kg per day from dialysis fluids (Rönholm, Holmberg 2006, Hölttä et al. 2000b). If energy intake is lower than recommended, tube feeding or a gastrostomy should be considered. The macronutrient distribution of the diet is directed to be as follows: carbohydrate 45–65%, fat 30–40%, and protein 5–20% in children between one and three years. In children under one year these ranges should be as in basic baby formulas: carbohydrate 36–56%, fat 40–54%, and 7–12% protein (KDOQI Work Group 2009).

## **Protein supply**

A report by a joint Food and Agriculture Organization, the United Nations (FAO)/ the World Health Organization (WHO)/ United Nations University (UNU) expert consultation, has defined the safe levels of protein supply (minimum) for each age-group (safe levels 1.09–1.86 g/kg per day) (World Health Organization 1985). The recommendation of the Kidney Disease Outcomes Quality Initiative (KDOQI) Work Group for dietary protein intake in small children is the daily recommended supply for a healthy child plus roughly 0.3 g/kg per day extra to replace losses due to dialysis (KDOQI Work Group 2009, Quan, Baum 1996). For children under seven months this is 1.8 g/kg per day, for children under 12 months 1.5 g/kg per day, and for children under four years of age 1.3 g/kg per day (KDOQI Work Group 2009). In infants, supplemental nutrients are needed to avoid fluid overload and to maintain a sufficient amount of energy and protein (Rönnholm, Holmberg 2006).

## **Fats**

Suitable macronutrient distributions are given above. While cardiovascular diseases are the major cause of mortality and morbidity in children with ESRF the diet should be planned as to prevent increased plasma levels of triglycerides (TG) and cholesterol. If fat supplementation to formula is needed, it should consist of unsaturated fats such as in canola, olive, soybean, or rape-seed oils. High amounts of cholesterol, saturated fat, or trans fatty acids in the diet may raise total cholesterol and low-density lipoprotein (LDL) cholesterol levels in blood. On the contrary, high dietary intake of polyunsaturated fatty acids such as eicosapentanoic acid (EPA), omega-3 fatty acids, and docosahexanoic acid (DHA) decrease TG levels in plasma. In case of hyperlipidemia (high LDL), the proportion of fat should be lower than usual (<30% of calories), trans fatty acids should be avoided and saturated fatty acids should not exceed 7% of the calorie amount of the daily diet. In patients with high TG, in addition to low dietary fat and emphasis on polyunsaturated fatty acids, only a low quantity of simple carbohydrates should be served and complex carbohydrates favored (KDOQI Work Group 2009).

## **Micronutrients and vitamins**

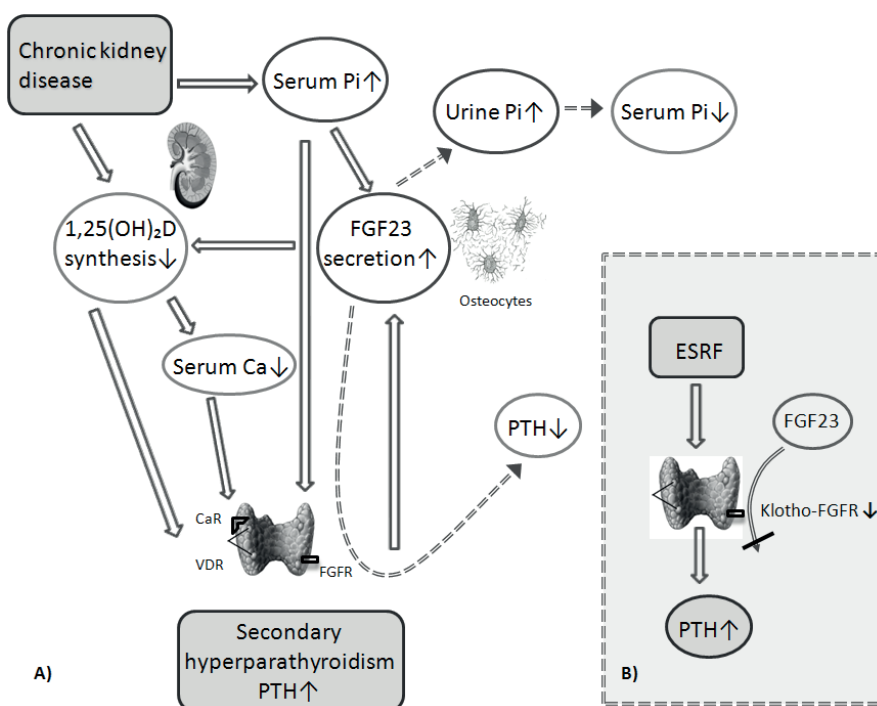
Very little is known about the vitamin and micronutrient requirements of children with chronic renal failure and during PD (KDOQI Work Group 2009, Rees, Shaw 2007). In adult patients on PD, it has been shown that substitution with some water-soluble vitamins (C, B vitamins) is necessary to avoid vitamin deficiencies (Kopple et al. 1981, Ross et al. 1989, Blumberg et al. 1983). Dialysate losses are partly responsible for the increased need of these vitamins (Blumberg et al. 1983, Mydlik et al. 1985). In children on PD, the blood concentrations of water-soluble vitamins have been shown to meet or exceed normal values with combined dietary and substitution intake (Kriley, Warady 1991, Warady et al. 1994). It has been suggested that children with chronic kidney disease should be given the same amount of vitamins as well as

micronutrients and minerals as is recommended for healthy children (Rees, Shaw 2007). The KDOQI Work Group recommends offering 100% of the daily recommended intake for children with ESRF. If the dietary intake is below this goal, supplementary water-soluble vitamins should be given. Fat-soluble vitamins are normally not needed in addition to food intake if the patient stays on the planned diet (KDOQI Work Group 2009). In children on PD, low serum levels of copper, selenium, and zinc have been documented (Warady et al. 1994, Tamura et al. 1989, Zachara et al. 2006). KDOQI recommends that the levels of copper should be monitored every 4 to 6 months, and the recommended daily intake of copper, selenium and zinc should be provided (KDOQI Work Group 2009).

### 2.2.1.2 Medication

#### Treatment of secondary hyperparathyroidism

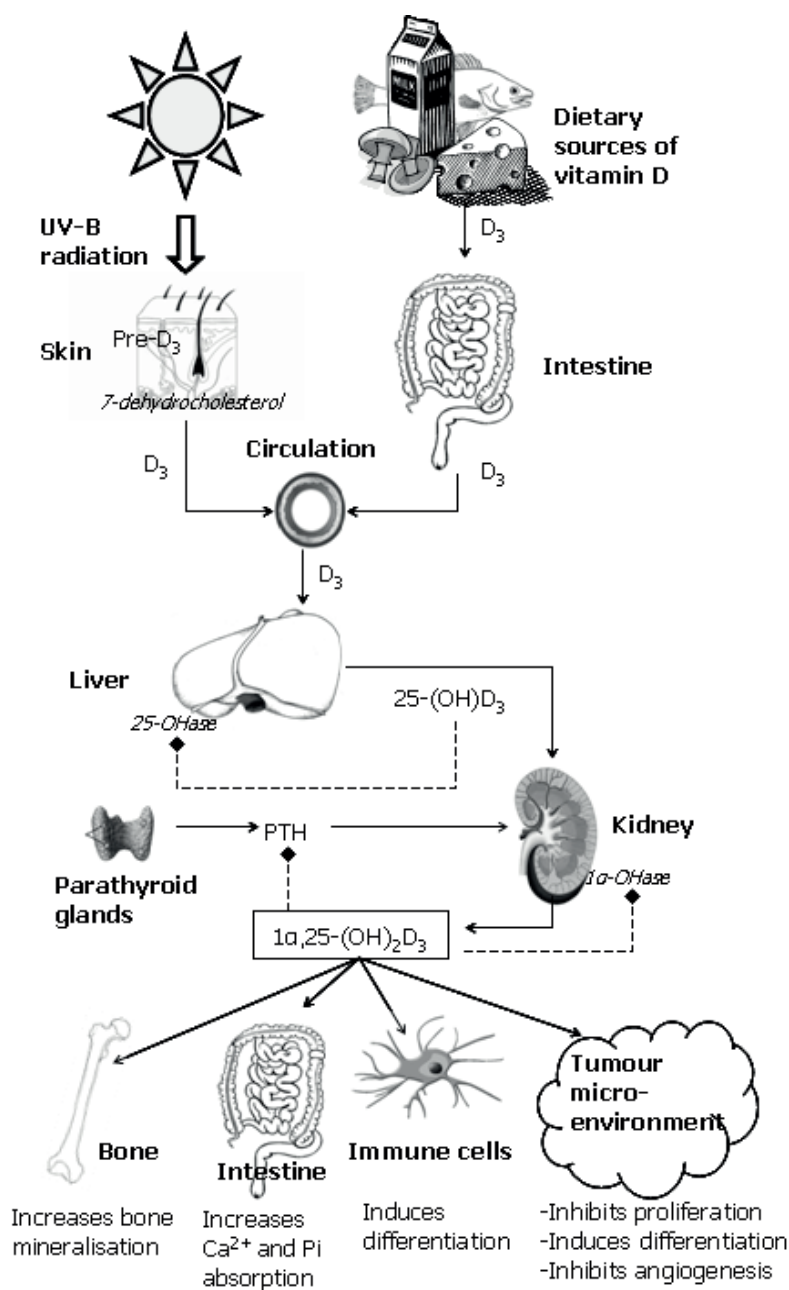
Secondary hyperparathyroidism is developed as a sum of many factors in chronic kidney disease (CKD) (see Figure 3). Treatment of hyperphosphatemia and hypocalcemia as well as supplementation with vitamin D in children with ESRF, are discussed in the next chapters.



**Figure 2** A) Pathogenesis of secondary hyperparathyroidism in chronic kidney disease. Phosphate (Pi), calcium (Ca), serum vitamin 1,25(OH)<sub>2</sub>D, serum parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), FGF23 receptor (FGFR), calcium receptor (CaR), vitamin D receptor (VDR). The pathways that are disrupted in ESRF are marked with broken line arrows. B) In advanced chronic kidney disease such as ESRF, the Klotho-FGFR complex is down regulated. Remodeled after Isakova and Wolf (2010) and Silver and Naveh-Many (2010).

### *Hyperphosphatemia*

Hyperphosphatemia caused by diminished excretion of phosphate in the kidneys is first treated with dietary phosphate restriction. Traditional medications for hyperphosphatemia are calcium carbonate ( $\text{CaCO}_3$ ) products, which bind to phosphate and reduce absorption of food phosphate in the intestine. At the same time, the patients receive calcium for their hypocalcemia. However, vascular calcification and higher incidence of cardiovascular disease compared to the general population have been reported in adults, as well as in childhood-onset ESRF (Querfeld 2004, Oh et al. 2002, Groothoff et al. 2005). Cardiovascular diseases are also a much more common cause of death compared to malignancies in patients transplanted during childhood (Offner et al. 1999). Sevelamer hydrochloride is used as a calcium free phosphate binder in grown-up patients, however in children, and especially in infants, sevelamer hydrochloride is scarcely used (Rees, Shroff 2010). Sevelamer hydrochloride often produces metabolic acidosis, which can be avoided by using sevelamer carbonate (Gonzalez et al. 2009). Nonetheless, today there are no calcium-free phosphate binders licensed for children (Rees, Shroff 2010) and the Kidney Disease Outcomes Quality Initiative (KDOQI) recommends that if sevelamer is used as the only phosphate binder in children, calcium should be supplemented with a calcium-including phosphate binder or/and a higher calcium concentration in the dialysate should be used (National Kidney Foundation (KDOQI) 2005).



**Figure 3** Vitamin D and parathyroid hormone metabolism. Modeled after Deeb et al. (2007). Negative feedback is indicated with a dashed line and diamond.

### *Vitamin D substitution*

The effects and metabolism of vitamin D in a healthy person are shown above in Figure 3. Vitamin D deficiency in ESRF originates from reduced or absent renal  $1\alpha$ -hydroxylation of vitamin 25-OH-D (calcidiol), which in healthy people is already 25-hydroxylated in the liver. Because of the  $1\alpha$ -hydroxylation defect, ESRF patients need vitamin D substitution as  $1\alpha$ -hydroxylated,  $1\alpha$ -hydroxycholecalciferol (alphacalcidol) which is then hydroxylated in the liver to its active form 1,25(OH) $_2$ D (calcitriol), which may also be used as vitamin D substitute. Calcitriol increases the absorption of calcium and phosphate in the gut, increases bone mineralization and adjusts the level of serum calcium. Calcitriol provides negative feedback to PTH excretion thereby inhibiting the high bone turnover caused by high PTH. Providing an accurate dosage of alphacalcidol is challenging, because too high doses reduce PTH secretion, and over-suppression of PTH leads to adynamic bone disease, in which bone turnover is too low (Wesseling et al. 2008). Thus, to ensure good growth, the European Paediatric Dialysis Working Group recommends that in ESRF patients, the aimed level of plasma PTH should be two to three times the upper normal limit (Klaus et al. 2006).

### **Treatment of anemia**

Erythropoietin (EPO) deficiency or relative deficiency, alterations in the activity of EPO caused by uremic toxins, and inhibition of erythroid progenitor cell formation in bone marrow are the most important factors causing anemia in children with ESRF (McGonigle et al. 1984, McGonigle et al. 1985). Regular erythropoietin injections and supplemental oral iron form the basis of anemia management in children with ESRF. In HD patients, the use of intravenous iron is preferred (KDOQI, National Kidney Foundation 2006). Infants require higher doses of EPO, from 275 to 350 U/kg per week, compared to older children and adults. PD patients need less EPO than HD patients (NAPRTCS 2004 Annual Report 2005). Early EPO therapy has been associated with improved growth in children with chronic kidney disease (Boehm et al. 2007).

### **Treatment of hypertension**

Hypertension has been observed in 55% of children during dialysis in Poland. The mean age of these patients was  $10 \pm 5$  years and the hypertension incidence rate was similar in boys and girls (53 vs. 60%) as well as in PD and HP patients (54 vs. 56%). Monotherapy was used in 33% and 36% received two antihypertensive drugs (Tkaczyk et al. 2006). In comparison, systolic hypertension was seen in 52% of Finnish children on PD and diastolic hypertension in 43%. Furthermore, 73% of children below five years of age had systolic hypertension. The antihypertensive medicines used in Finnish patients were mostly calcium channel blockers and  $\beta$ -blockers (Hölttä et al. 2001).



## 2.2.2 Active treatment - dialysis

When renal function is severely reduced or absent, the tasks typically performed by the kidneys must be substituted. Their purification function and removal of excess water from the body are both managed by dialysis. Peritoneal dialysis (PD) and hemodialysis (HD) are common treatment possibilities, although hemodiafiltration may also be used in mainly acute situations.

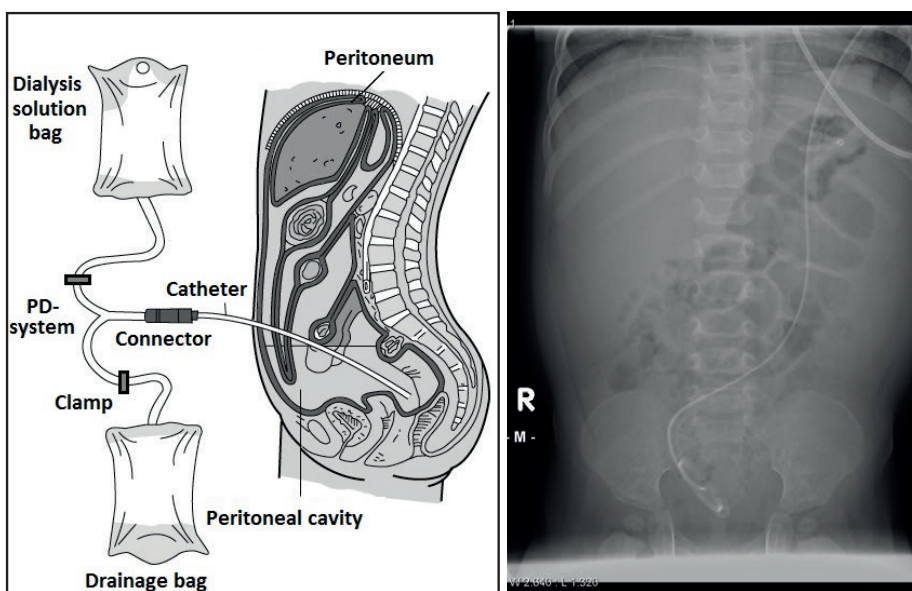
In European ESRF patients under 5 years of age the first treatment modality has been PD in 70%, HD in 26% and pre-emptive renal transplantation in 4% according to the European Renal Association – European Dialysis and Transplantation Association (ERA-EDTA) report of 2004 (van der Heijden et al. 2004). In Finland, the percentages for the same age group between 1990 and 2008 were 97% for PD, 1% for HD and 2% for pre-emptive transplantation (Finnish Registry for Kidney Diseases 2009).

The emphasis of this study is on PD treatment, and HD is discussed only briefly.

### 2.2.2.1 Peritoneal dialysis

Peritoneal dialysis has been used in children since the late 1970's, starting in Canada (Oreopoulos et al. 1979). In Europe PD was later introduced and became an established treatment modality in children in the 1980's (Alexander, Honda 1993). During PD, a dialysis solution (dialysate) is infused into the patient's peritoneal cavity through a PD catheter and left there for a prescribed dwell time. The peritoneal membrane works as a semi-permeable filter through which water as well as urea, potassium, phosphate and other waste products diffuse from blood in the capillaries of the peritoneum into the dialysate to a lower concentration. Waste material is removed from the body by changing the dialysis solution several times per day to maintain the concentration gradient. The dialysate contains glucose, which creates an osmotic gradient between blood and dialysate. With this gradient, excess water is removed from the body. Small molecules such as urea move faster through the peritoneal membrane compared to larger molecules such as creatinine and phosphate (Gao et al. 2004).

The dialysate can be exchanged manually or with the help of a dialysis machine. There are different kinds of PD machines with modern techniques, programs, and tubing suitable also for small children. At this moment Sleep●safe™ (Fresenius Medical Care, Bad Homburg, Germany), HomeChoice Pro (Baxter Healthcare Corp., Deerfield, IL, USA) and Serena® (Gambro AB, Stockholm, Sweden) are suitable machines for infants.



**Figure 4** A) Principle of peritoneal dialysis. Adapted from Picsicio (<http://picsicio.us/image>). B) X-ray image showing the right position of a PD catheter in the peritoneal cavity.

### Access of PD

The access for PD, the catheter, is set into the peritoneal cavity through the abdominal wall. The implantation is recommended to be performed approximately two weeks prior to starting dialysis treatment, especially in children, to avoid leaks (Rönnholm, Holmberg 2006). Implantation should be carried out by experienced surgeons (Watson et al. 2001). According to the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) report 2007, most PD catheters used were the Tenckhoff curled (62%) or Tenckhoff straight (27%). Single-cuffs were present in about half of the catheters (53%), whilst 65% had a straight tunnel, and 40% of the exit-sites had a lateral orientation. There was no difference in the incidence of peritonitis between different catheter properties (NAPRTCS 2007 Annual Report 2008). Furth et al. also found no clear difference between single and double-cuff catheters concerning exit-site or tunnel infection frequency. Nor was there any difference in exit-site and tunnel infection incidence between coiled and straight catheters or between different exit-site orientations (up/down/lateral) (Furth et al. 2000).

### Modalities of PD

The first modality of PD was continuous ambulatory peritoneal dialysis (CAPD), which was replaced over time by continuous cycling peritoneal dialysis (CCPD); this first automated peritoneal dialysis form was developed to reduce the frequency of peritonitis (Price, Suki 1981). Tidal peritoneal dialysis (TPD) was introduced some years later; in TPD, after the initial fill volume is instilled, a fraction of the volume is exchanged

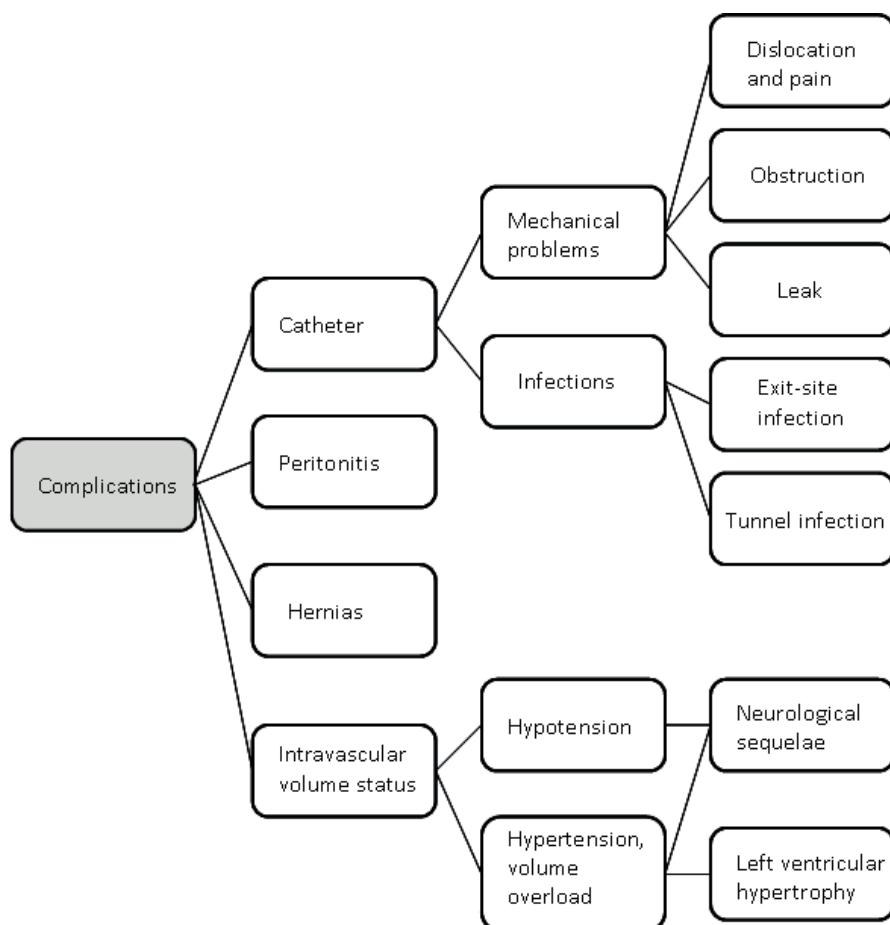
frequently using a cycler machine (Flanigan et al. 1991). It may provide advantages for children with outflow problems or outflow pain, and allows better purification for patients with high peritoneal membrane permeability (Hölttä et al. 2000a).

Automated peritoneal dialysis (APD) is performed overnight, the treatment time varying from 8 to 12 hours with 8 to 14 exchanges per night in CCPD. The fill volume in infants is usually 800—1000 ml/m<sup>2</sup> of BSA per exchange (Rönnholm, Holmberg 2006) and in older children larger volumes, from 1000 to 1200 or even up till 1400 ml/m<sup>2</sup> of BSA, are recommended (Fischbach, Warady 2009). The last fill left in the peritoneal cavity in the morning is usually half of the nightly fill volume on order to avoid discomfort, vomiting, and development of hernias. In addition to automated peritoneal dialysis (APD), anuric infants also need extra daytime exchanges to avoid hypervolemia (Rönnholm, Holmberg 2006).

### **Adequacy of PD**

The adequacy of peritoneal dialysis is usually measured with a dialysate and urine collection test, from which Kt/V for urea and creatinine clearance (Crcl) are calculated (II. NKF-K/DOQI 2001). Kt/V urea was initially developed to illustrate the adequacy of hemodialysis but was later embraced for PD purification evaluations (Bargman 2006). In Kt/V, K represents clearance, t stands for observed time period (here 24 hours) and V for the volume of distribution (total body water). Thus, the clearance of urea is compared to total body water (TBW), in contrast to creatinine clearance which is compared to body surface area (BSA). Crcl describes the amount of creatinine that is removed from the blood over a time interval. According to the National Kidney Foundation (NKF) Work Group, based on the available evidence, the minimum target of weekly urea Kt/V should be 2.0 and of Crcl, 60 L/week per 1.73 m<sup>2</sup>. If there are difficulties in achieving these targets, the Kt/V for urea should be the principle measure of adequacy. Urea Kt/V directly reflects protein metabolism and is also less influenced by alternations in residual renal function (II. NKF-K/DOQI 2001). These measures characterize small and middle size molecule purification. Moreover, blood urea nitrogen, blood creatinine and phosphate are measures of purification; the lower these measures are, the better the purification is thought to be. The peritoneal equilibration test (PET) expresses peritoneal membrane function (Twardowski et al. 1987) and together with it and dialysate and urine collection tests it is possible to predict urea and creatinine clearances also in pediatric patients (Verrina et al. 1998, Warady et al. 2001).

### 2.2.2.2 Complications of peritoneal dialysis



**Figure 5** Complications of PD.

#### 2.2.2.2.1 Catheter-related complications

Catheter-related mechanical problems and infections are noteworthy causes of morbidity and treatment failure in children on PD, despite the advanced catheter models and PD techniques (Furth et al. 2000, Macchini et al. 2006). In 1995, the NAPRTCS reported the revision of 20% of the catheters in children of all ages (Neu et al. 1995), and in 2007 the revision of 19% (NAPRTCS 2007 Annual Report 2008). In a recent Italian study in children, where the patients' mean age was six years, the catheter survival rate was 80% at one year, and 62% at two years on PD, and the incidence of catheter-related complications was one episode per 6.4 months on PD when peritonitis was included. Catheter survival was longer in children over five years compared to children less than two years of age. Single-cuff catheters had lower infection rates

compared to double-cuff catheters (Macchini et al. 2006). Aksu et al. (2007) have reported a very low catheter complication rate of 1 episode per 24.7 patient-months and a high catheter survival rate of 92% at one year and 83% at 2 years in children between three months and 16 years (mean age eight years).

### *Mechanical problems*

In the above mentioned Italian study, catheter obstruction constituted 13%, dislocation 9%, and leakage 6% of the complications when peritonitis was excluded (Macchini et al. 2006). In the NAPRTCS report of 2007, malfunction comprised 48% and leak 5% of complications also when peritonitis was not included (NAPRTCS 2007 Annual Report 2008). In the report of Aksu et al. (2007), dislocation yielded 9%, leakage and rotation both 5% of the complications, again when peritonitis was excluded. Omentectomy has been shown to reduce obstruction rate and lengthen the survival time of the catheter (Macchini et al. 2006, Reissman et al. 1998, Rinaldi et al. 2004). The surgical technique and an experienced team for catheter placement are the most critical factors for catheter survival (Macchini et al. 2006, Aksu et al. 2007).

### *Exit-site and tunnel infections*

Exit-site infection diagnosis should be made if there is a purulent drainage from the exit-site, or notable pericatheter edema, redness, or/and tenderness locally. A positive bacterial culture is not required for the diagnosis of exit-site infections (Table 2, Schaefer et al. 1999b, Warady et al. 2000).

In a recent Italian study, exit-site infections (ESI) and tunnel infections (TI) were shown to be responsible for 59% of catheter-related complications when peritonitis was excluded (Macchini et al. 2006), whilst in the NAPRTCS report of 1995, ESI or TI was seen in 36% of children between 0 and 5 years (Neu et al. 1995). In contrast, the NAPRTCS report of 2007 demonstrated that ESI and TI comprised 18% of catheter-related complications when peritonitis was excluded (NAPRTCS 2007 Annual Report 2008). In the report of Aksu et al. (2007), ESI and TI proved to be the most common catheter complications (81% when peritonitis was not included), and these were more frequent in younger patients. On the other hand, Furth et al. (2000) had stated earlier that catheter characteristics, patient age, or race, had no influence on the risk of ESI or TI.

**Table 2.** Exit-site infection scoring system. A score of  $\geq 4$  means infection. Purulent drainage, even alone, is an evidence of infection. Adapted from Schaefer et al. (1999b) and Piraino et al. (2005).

	0 points	1 point	2 points
Swelling	no	exit only; $<0.5$ cm	$>0.5$ or/and tunnel
Crust	no	$<0.5$ cm	$>0.5$ cm
Reddness	no	$<0.5$ cm	$>0.5$ cm
Pain	no	slight	severe
Drainage	no	serous	purulent

#### 2.2.2.2.2 Peritonitis

In children, fever and abdominal pain are considered too nonspecific as symptoms for the diagnosis of peritonitis. The diagnosis should be made if the dialysis effluent is cloudy, the white blood cell count is more than 100 per  $\mu\text{L}$  in the effluent, and at least 50% of the leucocytes are polymorphonuclear (Warady et al. 2000).

Peritonitis is a very potent cause of morbidity and the most important cause of PD failure despite reduced incidence of infections over the years (Chadha et al. 2010, Schaefer et al. 2007). Many reports demonstrate a higher incidence of peritonitis in younger children (Neu et al. 2002, NAPRTCS 2007 Annual Report 2008, Hölttä et al. 2000b, Honda et al. 1996, Hoshii et al. 2006). The NAPRTCS report of 2007 shows an annualized peritonitis rate of 0.79 (number of peritonitis per year) between 1992 and 1996 and a rate of 0.57 between 1997 and 2006. In children less than two years of age the rate during the whole follow-up period was 0.86 compared to 0.61 in children over twelve years (NAPRTCS 2007 Annual Report 2008). A recent report from Germany showed a peritonitis incidence of 0.85 episodes per patient-year in infants starting PD during the first year of life (Wedekin et al. 2010). Other risk factors for peritonitis are existing ESI or TI of the catheter, a higher degree of connections and spiking of the dialysate bags, and not using prophylactic antibiotics at the time of catheter placement (Chadha et al. 2010, Szeto et al. 2001, Gadallah et al. 2000).

Peritonitis is most commonly caused by bacteria, in particular gram-positive bacteria, that caused approximately 50—70% of the episodes in the early days of PD. With improved treatment protocols, the incidence of Gram positive peritonitis has been distinctly reduced, whilst the overall incidence of peritonitis has decreased. Better connection technology on PD, prophylactic medication for nasal carriage of *Staphylococcus aureus*, good exit-site care, improved skills of the medical team, and training of the family are partly responsible for the decreased peritonitis rate (Macchini et al. 2006, Chadha et al. 2010, Piraino et al. 2003). The causative organisms of peritonitis, according to recent studies, are shown in Table 3.

**Table 3.** Causative organisms of peritonitis in children on PD.

	IPPR			NAPRTCS	
	Warady et al. 2007	Aksu et al. 2007	Macchini et al. 2006	Furth et al. 2000	Hölttä et al. 2000
Patient amount; n	392	93	78	1258	10<5 yrs, 11<15 yrs
Patients age, years; median (range)	10 (0-22)	(0.25-16)	nd	(0-21)	(0.3-2.3), (4.8-14.4)
Patients age, years (mean $\pm$ 1 SD)	nd	8 $\pm$ 4.2	6.3 $\pm$ 6.1	nd	1 $\pm$ 0.6, 9.6 $\pm$ 3.4
Incidence; peritonitis per patient month	nd	1 per 18.1	1 per 13.2	1 per 13.2	total: 1 per 11.4 1 per 9.4, 1 per 15.8
Gram-positive	44 %	nd	nd	49 %	72 %
Coagulase negative Staphylococcus	17 %	2 %	46 %	nd	6 %
Staphylococcus aureus	15 %	9 %	14 %	nd	44 %
Streptococci	6 %	2 %	nd	nd	6 %
Enterococci	4 %	nd	5 %	nd	6 %
Gram-negative	25 %	nd	nd	22 %	22 %
Pseudomonas	6 %	9 %	8 %	nd	0 %
Klebsiella	5 %	2 %	8 %	nd	6 %
Escherichia coli	4 %	6 %	nd	nd	6 %
Fungi	2 %	nd	4 %	2 %	0 %
Culture negative	31 %	63.5%	nd	22 %	6 %

IPPR = International Pediatric Peritonitis Registry

NAPRTCS = North American Pediatric Trials and Collaborative Studies

nd = no data

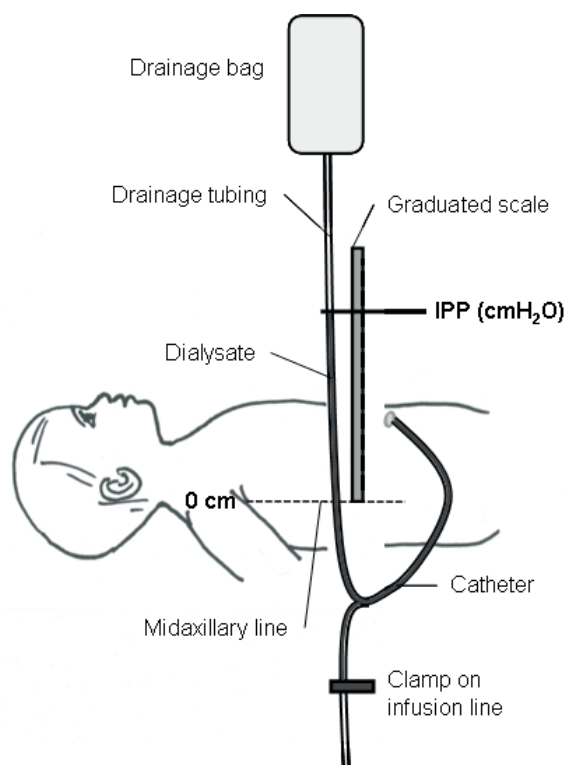
### 2.2.2.2.3 Hernias

Hernias are frequent in PD patients, especially in small children, although the existing data is scarce concerning infants. Earlier studies report hernias in 23 to 40% of children of all ages, mostly inguinal and ventral ones (Hölttä et al. 1997, von Lilien et al. 1987, Khoury et al. 1991, Verrina et al. 1992). One later study reports hernias in 11.5% of children with a mean age of nine years at PD onset (Donmez et al. 2003). Very few recent studies of children on PD report a prevalence of hernias.

#### *Intraperitoneal pressure*

Aranda et al. showed that intraperitoneal pressure (IPP) influences the frequency of hernias, and that IPP is lower in children compared to adults (Aranda et al. 2000). Elevated IPP can cause discomfort, pain, gastrointestinal reflux, hernias, and also loss of ultrafiltration. IPP measurement is an important tool in prescribing dialysis treatment concerning tolerance and adequacy (Fischbach et al. 2003). Durand et al. first presented the IPP measurement technique in adults (Durand et al. 1992) and Fischbach later applied it in children (Fischbach et al. 1994, Fischbach et al. 1996, Fischbach et al. 1996). The measurement itself is simple: the child rests in a supine position, the zero level is set in the patient's mid-axillary line and the mean value of inspiration and expiration is used (see Fig.6) (Durand et al. 1992). Fischbach et al. recommend a normal value for IPP of less than 18 cm of water, although between 5

and 15 cm of water is acceptable, depending on the fill volume. The patient's age, sex, and body mass index should also be taken into account (Fischbach et al. 2003).



**Figure 6** IPP measurement. Adapted from Durand et al. (1992).

#### 2.2.2.2.4 Intravascular volume status

##### *Hypertension*

Hypertension is a common problem seen in as many as 52 to 63% of children on PD (Tkaczyk et al. 2006, Hölttä et al. 2001, Donmez et al. 2003, Acar et al. 2008, Mitsnefes et al. 2003, Mitsnefes, Stablein 2005). According to NAPRTCS, 77% of the patients (both PD and HD) suffered from hypertension, of which 57% was uncontrolled and 20% controlled at the beginning of dialysis. Risk factors for hypertension included high blood pressure (BP) at baseline, young age, and acquired kidney disease as a cause for ESRF, and HD as dialysis mode. BP remained high in most patients during the first year on dialysis and medication did not correct the situation. However, the first months on dialysis seemed to be crucial for getting BP under control (Mitsnefes, Stablein 2005).

##### *Hypovolemia*

Hypotension and hypovolemia during dialysis may cause permanent brain or nervous system damage. Early cases in Finland reported severe hypovolemia during dialysis



in small PD patients (Qvist et al. 2002, Valanne et al. 2004). One of these patients, with NPHS1, that experienced longer hypotensive periods, acquired permanent, severe visual impairment. There have been three reports on small children aged 1 to 5 years with anterior ischemic optic neuropathy as a consequence of hypovolemia or a period of low blood pressure values during PD. A permanent visual handicap was reported for all, although their hypovolemia and blood pressure levels were corrected (Bates et al. 1999, Chutorian et al. 2002, Lapeyraque et al. 2003). Two patients also had changes in their brain magnetic resonance imaging (MRI): one displayed periventricular white matter abnormalities and the other encephalomalasia and gliosis in the occipital cortex bilaterally. These changes suggest chronic hypoperfusion (Bates et al. 1999, Lapeyraque et al. 2003).

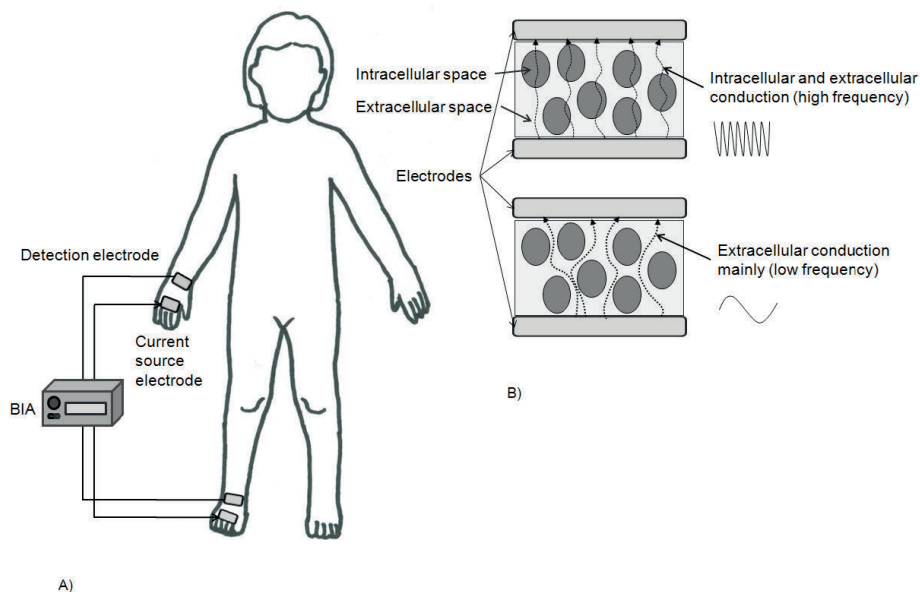
#### *Heart ultrasound measures*

Cardiovascular diseases are a significant reason for deaths and accounted for 32% of the deaths in children and young adults with ESRF in a single center study (Offner et al. 1999). Left ventricular hypertrophy (LVH) is often detected (45 to 75%) in pediatric PD and HD patients (Hölttä et al. 2001, Civilibal et al. 2009, Mitsnefes et al. 2000). Left ventricular mass index (LVMI = left ventricular mass divided by body height<sup>2.7</sup>) was also higher in children on dialysis compared to a control population in a couple of studies, reflecting a more frequent prevalence of LVH in dialysis patients (Robinson et al. 2005, Ten Harkel et al. 2009). Hölttä et al. found that children under 5 years of age displayed more frequent LVH when compared to older children (60% versus 30%) (Hölttä et al. 2001). In many studies on children with ESRF, the association between LVH (or high LVMI) and elevated blood pressure has been established (Hölttä et al. 2001, Ten Harkel et al. 2009, Mitsnefes et al. 2003, Mitsnefes et al. 2001). There are studies indicating that volume overload and long-lasting hypertension further the development of LVH (Groothoff et al. 2005, Mitsnefes et al. 2003). In some studies the hemoglobin levels were lower in the patients with LVH compared to either patients without LVH or controls (Civilibal et al. 2009, Mitsnefes et al. 2000). Quantification of the left ventricular mass (LVM) is always an estimation which is based on formulas that fit ventricular shape to geometric figures (Vuille, Weyman 1994). Adjustment of LVM to body size can also be carried out in many ways such as by comparing LVM to height, BSA, or weight (Levy et al. 1987, Foppa et al. 2005). Additionally, defining the cut-off point between normal LVM compared to body size and LVH is a controversial matter and different methods yield diverse results regarding the prevalence of patients with LVH (Foppa et al. 2005).

#### *Bioelectrical impedance analysis*

Bioelectrical impedance analysis (BIA) is a non-invasive method to examine body composition and is based on different electrical properties of tissues (see Fig.7). First utilized in body fat evaluations, BIA is typically used to measure lean mass and body fluid volumes such as total body water (TBW). The quantities measured with BIA are reactance (Xc) that reflects body cell mass and resistance (R) that is inversely proportional to body water volume. The measurement can be done with a specific

electric current frequency or with several frequencies. At 50 kHz the current flows through both extra and intracellular fluid and BIA value correlates to TBW, but the value is in reality representing a weighted sum of extra-cellular water (ECW) and intra-cellular water (ICW) volume resistivities (Kyle et al. 2004, Ellis et al. 1999). Low frequencies do not pass the cell membrane as well as higher frequencies and thus better reflect the ECW (Deurenberg et al. 1995). To distinguish TBW, ECW and ICW, a multi-frequency device must be used (Kyle et al. 2004). An increased ECW or ECW/TBW ratio may be due to edema or malnutrition in a patient. Basile et al. (2007) have studied and developed dry weight prediction equations for adults on HD, whilst Edefonti et al. (2001) have used BIA in the evaluation of the nutritional status of children on PD. Accordingly, Wühl et al. (1996) have defined equations for calculating TBW in children on PD or HD at 50 kHz. The median age of these patients was 11.9 years (range 4.1—20.3). Later, Brooks et al. (2008) studied the correlation between BIA and TBW as well as BIA and blood pressure in older children on HD, although ECW evaluations or equations were not performed in these studies. The reference values of TBW and ECW for small children, especially for infants, do not yet exist which has restricted the use of BIA in small dialysis patients.



**Figure 7** A) BIA measurement, wrist and ankle placement of electrodes. Adapted from Chamney et al. (2002). B) Theoretical BIA current flow. Modeled after Chamney et al. (2002).

### 2.2.2.3 Mortality in children on peritoneal dialysis

The mortality of children on PD is typically 5 to 20% (Shroff et al. 2006, Hölttä et al. 1997, NAPRTCS 2007 Annual Report 2008, Hölttä et al. 2000b, Rees 2002).

The youngest patients have the lowest survival rate (Verrina et al. 2004, NAPRTCS 2007 Annual Report 2008). According to the NAPRTCS report from 2007, the most frequent causes of death were cardiopulmonary problems and different types of infection (NAPRTCS 2007 Annual Report 2008). Co-morbidities such as cardiac, gastrointestinal, and metabolic disorders were associated with 76% of the deaths in dialysis children in the study by Shroff et al. The most common causes of death were brain insults and various infections. The youngest patients were in greater risk for death; the mortality rate was almost threefold in children under five years of age compared to older ones (Shroff et al. 2006).

#### *2.2.2.4 Growth in children on peritoneal dialysis*

Growth in children with ESRF has been a significant concern since dialysis and transplantation in children began (Stefanidis et al. 1983). Between detection of renal disease and initiation of dialysis, growth deterioration has been demonstrated in most children in a study of Lewis et al. (2007). At the beginning of dialysis, 54% of the patients were under the fifth percentile in height Z scores (Lewis et al. 2007). In the NAPRTCS report of 2007, the mean height standard deviation score (hSDS) in children initiating dialysis was -2.55 in children less than two years of age, and -1.94 in children between 2 and 5 years. PD patients are smaller than HD patients and boys are proportionally smaller than girls at PD initiation (NAPRTCS 2007 Annual Report 2008). Several other studies confirm these results: in the report by Shroff et al. (2006), the mean hSDS at dialysis start was -2.8 in 98 children between birth and 16 years of age, whilst children under five had a mean hSDS of only -3.6 at initiation of dialysis. In the study by Cansick et al. (2007), a mean hSDS of -2.1 was seen in prepubertal children starting PD at a mean age of 2.8 years. Therefore, these children are already short statured when entering dialysis programs. Accordingly, the growth is poorest in young children.

Healthy children grow fast during the first two years, thus growth impairment in infancy has much consequence. Growth during dialysis has not improved as much as could have been expected with adequate nutrition and careful management of renal bone disease (Stefanidis, Klaus 2007). In a European multi-center study, over 18 months, the mean hSDS did not change notably during treatment (Schaefer et al. 1999a). The NAPRTCS reports changes of -0.11 and -0.15 in the mean height Z-scores one and two years after the initiation of dialysis for all children on dialysis. Only a minimal change of -0.07 in height Z-score was seen in PD patients one year after baseline and no change between one and two years was seen. In HD patients, changes of -0.18 and -0.31 one and two years after PD onset were detected (NAPRTCS 2007 Annual Report 2008). In the study of Cansick et al., catch-up growth was found in some patients less than 2 years of age during the first year of dialysis (Cansick et al. 2007). In a Finnish study, catch-up growth was observed in 62% of children less than 5 years of age during a 9-month period (Hölttä et al. 2000b). Adequate nutrition, mostly with dietary energy and protein supplementation via nasogastric tube or gastrostomy and strict management of phosphorus control are essential in

reaching good growth in children on dialysis (Cansick et al. 2007, Kari et al. 2000).

Recombinant human growth hormone (rhGH) is rarely used in children on PD, in only 16%, according to the European Dialysis and Transplantation Association (ERA-EDTA) Registry. Its use has not increased during the last years (Lewis et al. 2007). The NAPRTCS reported that 25% of PD patients and 14% of HD patients received rhGH during dialysis (Neu et al. 2002). Especially in infants, sufficient nutrition is the major factor in assuring growth. The management of hyperphosphatemia, hyperparathyroidism, and acidosis are crucial, as well. If the child with ESRF is small (hSDS <-1.88) or growth lags behind (height velocity <-2 SDS) despite optimal nutrition and medication, rhGH therapy should be started. An early administration of rhGH has been recommended but infants were not discussed separately (Stefanidis, Klaus 2007, Mahan et al. 2006).

It has been demonstrated that children with residual renal function (RRF) experience better growth on PD. Catch-up growth was seen in 58% of RRF patients compared to 17% of anuric patients in the study of Chadha et al.: about one third of their patients had rhGH treatment in both groups (RRF and anuric pt.) (Chadha et al. 2001). Poor growth has been associated with increased morbidity and mortality, where patients with growth failure display about a threefold risk of death compared to those with normal growth (Furth et al. 2002).

Target height (parent specific mean hSDS) takes the mean height of the local population and the height of the parents into account. It gives an estimate of the predicted height of the child and deviations of more than  $\pm 2$  SDS point to some disease or disturbance (Sorva et al. 1989).

#### *2.2.2.5 Hemodialysis*

Hemodialysis is less used in small children compared to older ones. Only 7% of ESRF patients under two years of age were treated on HD compared to 32% in all children with ESRF in North America between 1992 and 2007 (2006 Annual Report of NAPRTCS 2007). In Finland between 1990 and 2008, only 1% of children under two and 21% of all children with ESRF were treated with HD as first treatment mode (Finnish Registry for Kidney Diseases 2009).

In the USA, the vascular access of HD has been an external central catheter in 69–84% of pediatric patients since the 1990s (NAPRTCS 2007 Annual Report 2008). In Finland, HD access in children is typically a dual-lumen venous catheter in the jugular or subclavian vein. Arteriovenous (AV) fistulas have been the most common way of vascular access in Europe. However, especially in infants, the construction of an AV fistula is challenging (Fischbach et al. 2005). The blood is cleansed of waste products with extracorporeal perfusion by a HD machine. At the same time, excess water is removed by ultrafiltration. HD treatment is normally done at least thrice per week in infants (Fischbach et al. 2005). Between treatment days anuric and oliguric patients are restricted regarding their intake of liquids to avoid hypervolemia because only a limited amount of ultrafiltration can be achieved during each HD session. Intensified daily HD has been used and studied in children as well. Fischbach et al. suggested

in 2006 that daily HD could improve growth in children (Fischbach et al. 2006), and they later demonstrated that daily online hemodiafiltration dialysis, which includes both daily dialysis and convective flow added to HD, improves growth in children (Fischbach et al. 2010).

## **2.3 Neurological development and complications in children with end-stage renal failure**

### **2.3.1 Neuromotor function evaluations**

There are at least 15 distinct instruments with which neuromotor function can be evaluated in infants (Heineman, Hadders-Algra 2008). In Table 4, four different methods of assessment are presented, two of each kind. The age range of children that may be evaluated with these instruments varies from birth to four months and between 0 to 3.5 years. All these methods of assessment are aimed at distinguishing those infants developing within the normal range from the ones with a deviating neuromotor condition. These instruments differ not only in approach but also in validity and reliability. Three different validity characteristics, 1) constructive validity, that is the ability of a test to identify the measure that it proposes to identify, 2) concurrent validity, the degree to which a result from one test agree with a result from a different test, and 3) predictive validity, the ability to predict neurological outcome of the patient, are presented in Table 4. Intra and inter-observer reliability properties of these instruments are also shown in the table. The purpose of the assessment may be, 1) discriminative, to distinguish normal development from abnormal, 2) evaluative, to notice changes in the development or 3) predictive, to predict neurological outcome. Specifying the goal that the assessment must achieve is important for choosing the right method of assessment for each patient group and survey (Heineman, Hadders-Algra 2008).

**Table 4.** Different instruments for evaluation of neuromotor function in infants. Modified after Heineman and Hadders-Algra (2008).

Assesment	Age group	Purpose	Construct validity	Concurrent validity	Predictive validity	Intra- and inter-observer agreement
<b>Comprehensive neurological examinations</b>						
Touwen infant neurological examination	0 mo to independent walking	discriminative	no data	no data	good	no data
Hammersmith infant neurological examination	2 to 24 months	discriminative, predictive	good	no data	good	no data/very good
<b>Procedures with standardized scoring</b>						
Bayley scales of development 2nd/3rd edition	1 mo to 3.5 yrs	discriminative, evaluative	moderate	moderate	no data	no data/good
Neuromotor behavioral Inventory	0 to 12 months	discriminative	good	no data	no data	no data
<b>Observation of specific aspects of motor behaviour</b>						
Structured observation of motor performance	0 to 10 months	discriminative	good	no data	no data	moderate/good
Alberta Infant motor scale	0 mo to independent walking	discriminative	moderate	very good	moderate	very good
<b>Quality of motor behaviour and patterns</b>						
Test of infant motor performance	0 to 4 months (from 32 gest. wks)	discriminative	good	good	good	no data/good
General movements	0 to 4 months	discriminative predictive	very good	good	good	very good

### 2.3.2 Neuropsychological and cognitive performance tests

In older children, at least five years of age, different neuropsychological tests can be used to evaluate neurodevelopment and cognition. Fennell et al. and Crocker et al. used, among others, the Wide Range Achievement Test, Beery-Butenica Development Test of Visual Motor Integration to evaluate the neuropsychology of children with chronic renal failure (Fennell et al. 1990, Crocker et al. 2002). A Developmental Neuropsychological Assessment (NEPSY) and its extension NEPSY-II have been used for children with renal transplantation in Finland (Qvist et al. 2002, Haavisto et al. submitted). NEPSY can be used for children between 3 and 12 years. This instrument describes the patient's attention and executive functions such as self-regulation and flexibility in thinking, language and communication skills, sensor motor and visuospatial processing as well as memory functions and learning abilities (Korkman 1988). NEPSY-II is an extended version of NEPSY, can be used up to age 16 and also tests social perception (Korkman et al. 2007).

Intelligence tests have also been used for Finnish pediatric renal and heart transplantation patients (Qvist et al. 2002, Haavisto et al. submitted, Haavisto et al. 2010). These study groups used the revised Wechsler Intelligence Scale for Children (WISC-R), which is suitable for children aged seven or more and is a modern IQ test (Wechsler 1974). The Wechsler Preschool and Primary Scale of Intelligence, Editions I-III (WPPSI-I-III), has been developed after WISC and can be used in children between two and a half and seven years (Wechsler 2003). The same IQ tests were used by Fennell et al., Crocker et al., and Madden et al. in childhood chronic renal failure (Fennell et al. 1990, Crocker et al. 2002, Madden et al. 2003). However, these wider neuropsychological and IQ tests can not be used in infants.

Hellbrügge et al. have developed a Munich Functional Developmental Diagnostic test (MFED) to evaluate functional development in children between one to three years (Hellbrügge et al. 1978). MFED has been used to define early cognitive development in Finnish children exposed to alcohol during the fetal period (Autti-Rämö, Granström 1991a).

### 2.3.3 Neurological development

There are very few studies into the neurological development of infants on peritoneal dialysis. An early literature review by Polinsky et al. in 1987 showed developmental delay in 84% of children dialyzed in infancy. After successful renal transplantation, delay was seen in 31%. Aluminum load from prior phosphate binders, hyperparathyroidism, malnutrition, and psychosocial problems were suspected to influence development (Polinsky et al. 1987). Later, Honda et al. reported low developmental quotients at the end of PD in 69% of Japanese patients less than two years of age at PD initiation (Honda et al. 1995). Geary and Haka-Ikse showed better results in infants with ESRF, 41% of whom had developmental delay (Geary, Haka-Ikse 1989). Warady et al. reported neurodevelopmental delay at the age of one year in only 21% of children on PD. However in their study, patients with extra renal or neurological disease, as well as two sepsis patients who died before one year of age (18%), were not included in the



neurological evaluations. The neurological evaluations were made either with the Modified Developmental Assessment Test (MDAT) or the Bayley Scales of Infant Development (BSID) at one year. Less than 10%, two of the 28 evaluated patients, had significant extrarenal co-morbidity. Approximately two thirds of these patients were evaluated with the Stanford Binet Intelligence Scale or the Wechsler Intelligence Scale for Children (WISC) later, at least at 4 years of age, most of them having received renal transplants. In 79% of the patients, the intelligence was in the average range. Nineteen per cent of school aged children attended special education, one of these children was deaf (Warady et al. 1999).

Some studies have evaluated children dialyzed during the first years of life at a later age. Madden et al. stated that the intelligence quotient (IQ) (mean 87) was within the average range in 67% of children dialyzed in infancy and tested at the age of 1 to 12 years. Eighty-seven per cent of the patients were within two standard deviations of the norms (Madden et al. 2003). Qvist et al. showed a mean IQ of 87.5 in children transplanted before five years of age and dialyzed prior to renal transplantation, 42% being normal average. Moreover, another 42% of patients were classified as borderline to low average. Between 6 and 24% had a disorder according to neuropsychological tests (Qvist et al. 2002). Young ages at the onset of kidney disease, severity of the disease, and longer duration of the disease have been detected as risks for neurocognitive development in a study by Slickers et al.: these children with CKD, both dialysis and nondialysis patients, were studied after their seventh birthday but 59% of them had their kidney disease since birth (Slickers et al. 2007). Elzouki et al. found a clear correlation between malnutrition during the first two years of life and microcephaly in children with chronic renal failure. In their study, all developmentally delayed children, three out of fifteen, had microcephaly (Elzouki et al. 1994). Crocker et al. reported that children with congenital ESRF had worse results in long-term memory and fine motor coordination tests compared to children with acquired ESRF (Crocker et al. 2002). The child with a kidney disease and later ESRF may have co-morbidities such as a syndrome or neurological impairment such as hemiparesis. These co-morbid conditions naturally have an effect on the neurological outcome of the child (Offner et al. 1999).

Cerebral palsy (CP) is a descriptive term for a group of nonprogressive disorders of movement and posture caused by brain damage originating from pre or perinatal period or the first years of a child's life. CP includes changes in muscle tone (mainly rigidity or spasticity), muscle weakness, ataxia, unintentional movements or a combination of the above mentioned abnormalities. The extent of motor function deficit/abnormality may not be revealed before the age of 3 or 4 years (Swaiman, Wu 2006). Children with CP often also have other neurological problems such as mental retardation and speech and language disorders (Ashwal et al. 2004, Swaiman, Wu 2006). The prevalence of CP is typically between 1.2 and 2.5 cases per 1000 live births. Premature birth clearly raises the risk for CP; prevalence in very-low-birth-weight pre-term infants being 8 to 19% compared to that of 1.0 to 1.5% in full-term infants (Hagberg et al. 2001, Wu et al. 2003, Tommiska et al. 2003, Swaiman, Wu 2006).



### 2.3.4 Brain imaging

There are only few reports about brain imaging studies on dialysis and renal transplantation patients. In babies, the brain can be imaged with ultrasound (US), however, fontanels must be open for visibility. US is useful in discovering widened ventricles, hemorrhages, cystic changes and brain anomalies. However, US is not very sensitive in detecting hypoxic-ischemic brain damage (Rikalainen 2005). Computerized tomography (CT) is an ever less used method for studying the brain in children, except in acute trauma. The disadvantages of CT are poor tissue contrast and radiation stress. Nowadays, magnetic resonance imaging (MRI) is the principal method in studying the brain of a child. Hypoxic-ischemic lesions are easily detected with MRI although it may take a few days after the damage before changes are clearly visible. The alterations caused by ischemia in preterm and term infants and their development over time are well known. In small preterm infants, the fragile area for ischemia is the white matter around the brain ventricles. Over weeks and months, this damage converts into periventricular leukomalacia (PVL), in which the matter around ventricles becomes scarce and the ventricles become more angulated. The clinical condition of these children with PVL is typically spastic diplegia or tetraplegia. In full-term infants the susceptible areas to hypoxic damage are basal ganglia, the thalamus region and cerebral cortex. Longer and milder ischemia leads to watershed area infarcts (Valanne 2005).

Steinberg et al. performed brain CT in older children with either chronic renal failure, or PD treatment or renal transplantation (aged 2 to 18 years, n=22). None of those children had a co-morbidity affecting the central nervous system (CNS) and none had a clear CNS disorder at the moment imaging was done. Brain atrophy was present in 59% of the patients. A couple of patients had cortical infarcts and one had hypodensity in basal ganglia. Hemodialysis was found to be a risk factor for CT changes, but neither the type of renal disease, patient age, duration of renal failure, nor hypotension were related to brain atrophy (Steinberg et al. 1985). Elzouki et al. reported brain atrophy by CT in 23% of children with chronic renal disease from infancy (mean age 50 months at the end of the study, n=13) (Elzouki et al. 1994). Also Schnaper et al. observed brain atrophy by CT in 53% of children (n=15) with ESRF; they could not find clear risk factors but the HD treatment time of patients with brain atrophy was twice the time of patients without atrophy (Schnaper et al. 1983). Agildere et al. analyzed 432 adult patients that had received a renal transplant and 132 both child and adult patients that had received a liver transplant retrospectively with brain MRI. One third of the patients with renal Tx, and 72% of patients with liver Tx, displayed findings in their MRI after transplantation. The diagnoses were various, such as white matter changes, posterior reversible encephalopathy syndrome (PRES) owing to hypertensive encephalopathy or toxicity of immunosuppressive medication and intracranial hemorrhage. The findings were either related to transplantation or to chronic kidney or liver disease. Some findings seemed not to be secondary to the preceding conditions, but were considered to be incidental. The time on dialysis was not analyzed or discussed in the paper (Agildere et al. 2006). Another study by Valanne et al. defined neuroradiological findings in children with renal transplantation under five years of age (n= 33, born between 1984 and 1991).

MRI studies were done at a mean of six years after Tx, and most of these patients were on dialysis before Tx. Border-zone infarcts were recorded in 54% of patients; 11% of them severe, 33% moderate, and 56% mild lesions. Before transplantation, border zone infarcts were seen in 23% of the 26 patients imaged by CT (Valanne et al. 2004). These border zones are, for anatomical reasons, the most vulnerable areas to ischemia induced by hypoperfusion due to systemic hypotension (Adams et al. 1966) or serious stenosis of the internal carotid artery (Weiller et al. 1991). Hemodynamic crises, older age at transplantation, and longer duration of dialysis correlated well with border zone infarcts. Brain atrophy was seen in 15% of these children, cerebellar infarcts in 18%, and large vessel infarcts in 6%, after transplantation; however, 40% presented a normal brain MRI and 23% of them had had a clinical hemodynamic crisis (Valanne et al. 2004).

### 2.3.5 Hearing

In an early report by Bergström and Thompson, hearing loss was found in 47% of children with chronic renal failure. Ototoxic, congenital and genetic hearing defects were more common in children compared to older patients. The etiology of hearing loss was unknown in only 5% of the children (Bergström, Thompson 1983). Later reports showed hearing loss in 18 to 47% of children with chronic or end-stage renal disease (Qvist et al. 2002, Nikolopoulos et al. 1997, Mancini et al. 1996, Warady et al. 1993). Nikolopoulos et al. reported sensorineural hearing loss (SNHL) for an unknown cause in 30% of 46 children with chronic renal failure, whereas one patient (2%) had treatment with ototoxic antibiotics. None of the PD group (n=9) carried a hearing defect (Nikolopoulos et al. 1997). Aminoglycosides and furosemide often used in children with kidney diseases are responsible for ototoxicity (Matz 1990). In the study of Warady et al. (1993), four out of 14 pediatric patients (28%) on long-term PD, had hearing loss and three of them had received intra-venous aminoglycoside treatment. Mancini et al. found a significant correlation between aminoglycoside and furosemide treatments and SNHL in children with CKD (Mancini et al. 1996). The possible accumulation of drugs and diminished excretion of them due to renal failure in patients on PD must be taken into account during treatment.

## 2.4 Congenital nephrotic syndrome and neurological impairment

Nephrotic syndrome (NS) may be congenital, or it may develop later in life. It typically has many etiologies. In both types of NS, there are some conditions that have been associated with neurological problems (Goldenberg et al. 2005, Jalanko 2009).

Congenital NS, such as NPHS1, comprises a group of uncommon kidney defects in which severe proteinuria is present already from the first months of life. The cause of congenital NS may be genetic such as a mutation in *NPHS1*, or more frequently in *NPHS2*, causing nonfunctional protein podocin which is, like nephrin, a part of the slit diaphragm complex (Jalanko 2009). There are also other gene mutations such as *WT1*

in the Denys-Drash syndrome (Coppes et al. 1993), and *LamB2* in Pierson syndrome (Zenker et al. 2004b, Zenker et al. 2004a), causing proteinuria in the glomerulus. In Galloway-Mowat syndrome (GMS), in addition to NS, patients also present CNS anomalies including hypotonia, microcephaly, brain anomalies, and psychomotor retardation (Cooperstone et al. 1993, Kozłowski et al. 1989, Lin et al. 2001). It is an autosomal recessive disease, in which the genetic defect is unknown. In addition to NS and CNS impairment, these patients may display other extrarenal disorders such as short stature, facial anomalies and hiatus hernia (Jalanko 2009, Cooperstone et al. 1993, Kozłowski et al. 1989). Jalanko speculates that since podocytes and neuronal cells resemble each other and have common proteins in their structure there may be genetic disorders that affect both kidney and CNS (Jalanko 2009).

In late-onset NS, genetic defects of oxidative phosphorylation, that is defects in respiratory chain (RC); have been reported since the 1980s. These patients also have different neurological diseases, and some of them have hearing deficiencies. Their genetic defects have been detected in mitochondrial (mt) DNA (Tanaka et al. 1986, Hameed et al. 2001, Niaudet, Rotig 1996). Goldenberg et al. reported congenital NS with respiratory chain enzyme deficiency in a few patients in 2005. One of the three patients experienced seizures and a coma in the acute phase, but otherwise his neurology was normal. Two other patients died very young but their neurological abnormality was not described (Goldenberg et al. 2005).



### 3 Aims of the study

Infants and young children with ESRF have a poorer outcome, higher mortality, and suboptimal growth compared to older children. Due to *NPHS1* defects, more young children in Finland require treatment for ESRF, including PD, compared to other countries. Thus, we have an obligation to develop treatment modalities for this age group. PD is the most commonly used RRT modality in this age group wherein HD is more difficult to perform, especially if one wants the child to spend as much time at home as possible to improve development and quality of life for the child and his/her family.

In this study, we have focused on the youngest children, those needing PD before the age of two years. We have prospectively followed these children in detail using a standardized treatment and follow-up protocol based on our earlier studies and the literature, and compared the results with our previous experiences. We have also closely monitored the neurological outcome of these children.

The specific aims were:

1. To prospectively follow all children under 2 years of age at onset of peritoneal dialysis, using a standardized protocol, and evaluate their metabolic control, intravascular volume status, and growth.
2. To compare the results of prospectively followed patients with those reached during previous years.
3. To document neurological development and possible brain imaging pathology in these very young, still developing children.
4. To characterize a group of *NPHS1* children with similar neurological findings since birth, including muscular dystonia and athetosis.
5. To make improvements and new recommendations for our RRT therapy and follow-up based on the findings of these studies.



## 4 Patients and methods

### 4.1 Patients and controls

The studies included all children less than two years of age treated with chronic PD between 1995 and 2005 in Finland (studies I-III), and six NPHS1 patients with a neurological athetotic syndrome and their NPHS1 control group (study IV; see in detail Table 5).

**Table 5.** Demographic data of patients included in studies I to IV. In study IV the data of control patients is shown in parenthesis.

	I	II-III	IV
Patient number (controls)	23	21	6 (29)
Diagnosis leading to ESRF			
NPHS1	13	15	6 (29)
Cystic kidney diseases	4	2	-
Urethral valve	3	1	-
Hypoxic kidney injury	2	1	-
Syndromes	1	1	-
Fibromuscular dysplasia	-	1	-
Bilateral nephrectomy yes/no	17/6	17/4	6/0 (29/0)
Gender (male/female)	14/9	12/9	4/2 (18/11)
PD started between	1995–2000	2001–2005	1988–1998 (1984–1991)
Age at PD initiation (mean, years)	0.4 ±0.3	0.6 ±0.3	-
(range)	(0.01–0.8)	(0.0–1.3)	

ESRF = end-stage renal failure

NPHS1 = congenital nephrotic syndrome of the Finnish type

Study I included 23 small children who were treated consecutively with chronic PD between 1995 and 2000 in Finland. The inclusion criterion, in addition to PD treatment, was the child's age under two years at PD onset. Three patients also belonged to study IV. This retrospective data was analyzed in order to obtain a control group for our prospectively followed PD patients.

The prospective studies II and III included all small children born between 2001 and 2005 who were treated with a preplanned strict PD protocol. The inclusion criterion was also that the patient was aged less than two years at the commencement of PD. Study IV comprised NPHS1 patients with an unknown, congruent neurological

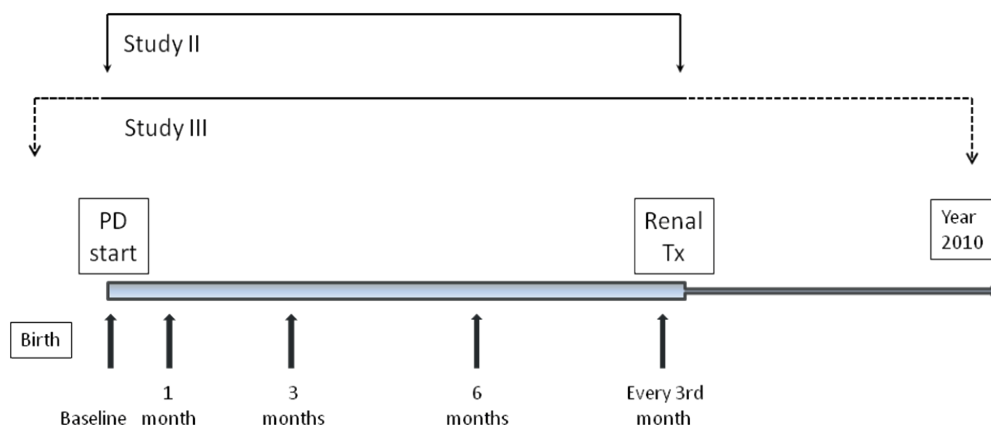
syndrome. Six children out of altogether 70 (8.6%) NPHS1 patients born between 1984 and 2003 had severe muscular hypotonia, dystonia, and athetoid movements. We call this movement disorder muscular dystonia and athetosis (MDA). Three of these patients were also included in study I.

As controls for NPHS1 patients with MDA, we enrolled 29 consecutive NPHS1 patients born between 1984 and 1991 who had undergone neurological and neuroradiological examinations for former studies at our institution (Qvist et al. 2002, Valanne et al. 2004).

## 4.2 Methods

### 4.2.1 Study design and data collection

In 2001, we made a prospective study protocol concerning PD treatment, nutrition, medication, and examinations in children under two years of age at the onset of PD. This study design is demonstrated below.



**Figure 8** Study design of the prospective part of this study in children with PD onset under two years of age (II, III). Ward visits are noted at baseline, one and three months and every third month thereafter.

The data from our retrospectively studied PD patient group (I) which included patient characteristics, information about the PD period and complications, medication, laboratory values, and growth data was collected from patient records and analyzed until the end of PD.

Methods of the study of NPHS1 with MDA patients (IV) are introduced later in this section (4.2.10).



### 4.2.2 Nutrition

Here and in the following chapters, we describe the methods used in our prospectively studied PD patient group unless otherwise stated.

Nutrition was planned by the same dietician and evaluated at least once per month, sometimes even weekly in patients with PD initiation between 2001 and 2005 (II). The energy amount of the diet was planned to be 80-90 kcal/kg per day, and protein provided 2.0-2.5 g/kg per day depending on the growth of the patient. The diet was mainly composed of infant milk and cereal products, and energy supplements. One patient required a gastrostomy and sixteen patients a permanent nasogastric tube to guarantee sufficient energy intake. Food intake was analyzed from two-day food records in 14 children. The Aivo2000-Diet32 program version 1.4.4.1. (AIVO Finland Oy, Turku, Finland) was used to count energy, protein, and nutrient amounts in the diet. We estimated that the children received additional energy from the dialysis solution glucose of approximately 10 kcal/kg per day. This amount was not included in the nutrition calculations.

### 4.2.3 Medication

Regular medication for ESRF was programmed for all patients (II). Calcium carbonate was used as a calcium substitute and phosphate binder. Alphacalcidol was given daily as vitamin D substitution, contrary to our earlier pulse therapy thrice a week used in the retrospectively analyzed patients. In case of a high, rapidly rising intact parathyroid hormone (iPTH) level of greater than 400 ng/L and high calcium-phosphate product (see below) the alphacalcidol dosage was switched to pulse therapy (Ala-Houhala et al. 1995).

All patients received subcutaneous human erythropoietin with an initial dose of 150 IU/kg per week and oral iron supplementation of 2-3 mg/kg per day to reach normal blood hemoglobin values. Medication is reported at baseline, and at three and six months. Comparison of the medication with the retrospectively analyzed PD patient group is done at six months. Antihypertensive medication was given to patients with hypertension when hypervolemia was excluded. Mainly  $\beta$ -blockers or calcium channel blockers were used. No one received growth hormone therapy during PD.

### 4.2.4 Dialysis

PD treatment initiation and family training took place in the Children's Hospital in Helsinki. For three patients, dialysis had been introduced already at their local hospital and chronic PD prescription and family training was performed at our clinic.

The dialysis catheter, mostly a Tenckhoff curled 1-cuff, in studies I and II, was inserted by the same two urologists at our center, the Children's Hospital in Helsinki, two weeks before PD initiation. Three children in the prospectively followed group (II) and seven children in the retrospective group (I) got their catheters only a few days before PD start, due to a prompt need of dialysis at their local hospital.

In the prospectively followed PD patient group (II), we used a nightly fill volume of

800 mL/m<sup>2</sup> of body surface area (BSA) per dialysis exchange in children under one year of age and 1000 mL/m<sup>2</sup> of BSA in older children. CCPD was the first choice of modality. Nightly PD treatment lasted approximately ten to eleven hours encompassing 12-14 exchanges. In nephrectomized patients, we performed two manual daytime dialysis solution exchanges (duration about one hour per exchange) to avoid hypervolemia. The last fill in the morning, as well as the daytime exchanges, were carried out with approximately 50% of the nightly dwell volume. TPD was chosen for patients with outflow pain, or ultrafiltration problems, or if better clearance was needed. TDP treatment time was the same as in CCPD but 22-26 exchanges with 50% dialysate exchange per cycle were performed during the night. In the middle of the TPD program one full outflow was performed to avoid overfill of the peritoneal cavity. In patients with PD initiation between 1995 and 2000 (I), the dialysis principle was similar to the one demonstrated above.

We used Baxter Home Choice cycler PD machines (Baxter Healthcare Corp., Deerfield, IL, USA) for all of our prospectively followed patients. Dialysates were pH-neutral bicarbonate-lactate-buffered dextrose solutions (Physioneal, Baxter Healthcare Corp.) and all dextrose concentrations were used. Weight limits were applied for the determination of glucose concentrations needed for exchanges. Nighttime PD was usually performed with low (glucose 13.6 mg/mL) and medium glucose concentration (22.7 mg/mL) mixtures but daytime exchanges were mostly carried out with high (38.6 mg/mL) or medium glucose concentration solutions to get better ultrafiltration with smaller daytime dwells. In a couple of patients that were growing poorly or with low plasma albumin values, we transiently used amino acid dialysate (Nutrineal, Baxter Healthcare Corp.) to improve their nutritional status. In one patient with ultrafiltration problems, we tried icodextrin dialysate without any notable help (Extraneal, Baxter Healthcare Corp.) before switching to HD. In retrospectively analyzed PD patients (I), other PD machines that are suitable for young children were also used.

#### **4.2.5 Uremia control**

All prospectively followed PD patients (II) visited our pediatric nephrology ward both one and three months after PD start, and every third month thereafter. Follow-up at their local hospital took place every one to three weeks. Growth measurements, laboratory tests, 24-hour dialysate and urine collections, IPP measurements, blood pressure, BIA, and heart ultrasound for the study were performed during these visits.

##### *4.2.5.1 Laboratory assessments*

Regularly followed routine laboratory tests are shown in Table 6.

Blood urea nitrogen (BUN) was targeted to remain below 40 mmol/L. Our target for blood hemoglobin was the lower limit of normal for age, at a minimum. Serum ferritin was aimed to be over 100 µg/L and saturated transferrin more than 20%. IPTH was aimed at high normal up to two-fold the upper limit of normal. Because the laboratory methods of iPTH varied during the study period, the upper limit of normal or multiples of it was used in reporting the values (the upper limit during the prospective study was

73 ng/L). Ionized-calcium phosphate product (ion-Ca x Pi) was targeted to be lower than 2.5 mol<sup>2</sup>/L<sup>2</sup> (Tertti, Klaus 2006).

**Table 6.** Laboratory assessments which were regularly examined in our prospectively followed patients during PD. Tests written in bold characters are reported in this thesis and/or in study II.

<b>Uremia control</b>	<b>creatinine (Crea)</b> <b>blood urea nitrogen (BUN)</b> sodium (Na) potassium (K) pH <b>bicarbonate (HCO<sub>3</sub>)</b>
<b>Hematopoiesis</b>	<b>hemoglobin (Hb)</b> and blood count iron (Fe) ferritin (ferrit) transferrin saturated transferrin (%)
<b>Calcium-phosphorus balance</b>	<b>ionized calcium (Ca-ion)</b> <b>phosphate (Pi)</b> <b>intact parathyroid hormone (iPTH)</b> <b>alkaline phosphatase (ALP)</b>
<b>Nutritional balance</b>	albumin (Alb) <b>prealbumin (Prealb)</b> <b>triglycerides (TG)</b> <b>cholesterols (total, HDL, and LDL)</b>
<b>Other</b>	<b>N-terminal atrial natriuretic peptide (ANP-N)</b> thyroid hormone (TSH) free thyroxine (T <sub>4v</sub> ) aspartate aminotransferase (AST) alanine aminotransferase (ALT) magnesium (Mg)

#### 4.2.5.2 Adequacy of peritoneal dialysis

An adequacy of dialysis was examined one and three months after dialysis initiation and thereafter every third month, coincident with the patient's normal PD prescription (II). We used 24-hour dialysate (and urine if not anuric) collection and analyzed it with the PD Adequest 2.0 computer program (Baxter Healthcare Corp.). Weekly urea Kt/V was targeted to be over 3.0. These children received more protein than adult patients and thus the urea purification was aimed higher than is recommended for

adults to guarantee that BUN values did not rise too high. Creatinine clearance (Crcl) was aimed to be more than 65 L/week per 1.73 m<sup>2</sup>. Crcl is related to BSA, which is relatively high in infants in comparison to older children and adults. The other method for reporting creatinine purification is weekly creatinine Kt/V, introduced by Ishikura et al. (2003), and it correlates the clearance of creatinine with total body water, not relative to BSA. We also calculated the weekly creatinine Kt/V in our prospective PD patients. According to Ishikura et al., in adult patients (male; 170 cm, 65 kg), a Crcl level of 60 L/week per 1.73 m<sup>2</sup> corresponds to a weekly creatinine Kt/V of 1.52 (Ishikura et al. 2003), which was used as cut-off point for acceptable creatinine purification in our patients too.

A 4-hour peritoneal equilibration test (PET) was performed directly after the 24-hour dialysate and urine collection with high glucose concentration dialysis solution and a fill volume of 800 mL/m<sup>2</sup> in children under one year of age and 1000 mL/m<sup>2</sup> in older children. The fill volume during PET was relatively low compared to the latest recommendations. However, in order to avoid high intraperitoneal pressure (IPP), we used high glucose concentration (38.6 mg/mL) solution in PET, which gives relatively high ultrafiltration (UF) and thus raises IPP during the test. The PET findings, the individual peritoneal transport capacities, are not reported here; however, PET results were used in the PD Adequest 2.0 program in planning an individual PD program for every patient and when checking it during ward visits.

A modified 24-hour dialysate and urine collection was used in the retrospectively analyzed PD patients (I) and it was available in only 15 patients (Hölttä et al. 2000b). It differs from the collection used in the prospective study. Thus, the comparison of purifications is not valid between these two patient groups.

## **4.2.6 Complications of peritoneal dialysis**

### *4.2.6.1 Catheter and hernias*

Data about catheter-related complications, and hernias were collected from patient records.

### *4.2.6.2 Intraperitoneal pressure measurement*

IPP was measured once or twice when introducing PD between 2001 and 2005, and then at least during every protocol ward visit (II). The dwell volume at measurement was the above mentioned 800 or 1000 mL/m<sup>2</sup> of BSA. To avoid discomfort, vomiting, and hernias and to be sure that the volume could be increased with growth in the near future, we targeted an IPP lower than 10 cm of H<sub>2</sub>O in children under two years of age, and lower than 16 cm of H<sub>2</sub>O in older children. If the IPP was clearly higher than targeted, we used dwell volumes smaller than the test volume on PD.

If clearances were poor (see below), and the IPP of an acceptable level, we increased the dialysis dosage by adding dialysis time, dwell volumes, or number of exchanges depending of the type of peritoneum.

#### *4.2.6.3 Peritonitis*

Peritonitis was considered possible when the patient had cloudy peritoneal fluid or clinical findings (fever, abdominal pain, diarrhea), and when the peritoneal fluid contained more than 100 leucocytes/ $\mu$ L, 50% or more of which were polymorphonuclear cells. A positive peritoneal fluid Gram stain amplified the diagnosis, and consequently, a positive peritoneal fluid or blood culture confirmed it (Thompson et al. 2005, Warady et al. 2000) (I, II).

#### *4.2.6.4 Intravascular volume status*

Blood pressure measurements were performed at ward visits. We used the mean value of oscillometric measurements taken every two hours over 24 hours. Weighing of the patient, plasma N-terminal atrial natriuretic peptide (ANP-N), BIA, and echocardiography were performed on the same day for intravascular volume status estimation (II).

### **Hypertension**

Our definition of hypertension was based on the Second Task Force reference values for diastolic and systolic daytime blood pressure. If the mean diastolic or systolic blood pressure exceeded the 95<sup>th</sup> percentile for age and gender, hypertension was established (National High Blood Pressure Education Program Working Group 1996). Patients with hypertension, signs of clinical overload, and ANP-N over 3 nmol/L, based on a previous study at our center (Hölttä et al. 2001), were regarded as hypervolemic (II).

In both patient groups, blood pressures were measured regularly, even daily, but accurate BP data was lacking in the retrospective patient group. Thus, we report hypertension frequency only in the prospectively followed PD patients (II) and comparison of the prevalence of hypertension between the two groups could not be made. See the medication issued for hypertension in chapter 4.2.4 (Medication).

### **Heart ultrasound measures**

All echocardiography examinations in the prospectively studied patients were performed by the same cardiologist (II). The data collected included, left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), interventricular septal dimension at end-diastole (SeptD), left ventricular posterior wall thickness at end-diastole (LVPWD), and ejection fraction (EF). The reference values for LVEDD, LVESD, SeptD, and LVPWD were computed according to Lester et al. (Lester et al. 1987). For these reference values, age, gender, height, weight, and heart rate (HR) were also taken into account. Left ventricular mass was defined using M-mode echocardiography and Devereux formula (Devereux et al. 1986). LVM was related to body height<sup>2.7</sup>, according to Simone et al., to establish a linear relationship (de Simone et al. 1992). This is cited here as the left ventricular mass index (LVMI). LVH was diagnosed if the LVMI exceeded the 95<sup>th</sup> percentile of gender and age (de

Simone et al. 1992). The LVM data were also analyzed according to Foster et al. with a new method in which LVM is related to body size to give an LV mass-for-height Z-score, which is appropriate for small children. LVH was also stated if the Z-score exceeded the 95<sup>th</sup> percentile. The Z-scores of LV mass-for-height and heart dimensions were taken to correlation analyses (II).

### **Bioimpedance analysis**

BIA measurements were performed with a Nutriguard-M multi-frequency analyzer (Data Input GmbH, Darmstadt, Germany) providing resistance (R) and reactance (Xc) (II). The patient was in supine position, with electrodes placed on hand and foot (Kyle et al. 2004). The electrodes were halved due to the small size of our patients. A frequency of 50 kHz was used in the measurements, which were technically demanding due to patient age. The resistance index  $RI = \text{height}^2/R$  (Houtkooper et al. 1989, Schaefer et al. 2000) was used for correlation analyses.

#### **4.2.7 Mortality and hospitalization**

Mortality is reported as deaths per follow-up years. Hospitalization is reported as the total number of days in hospital divided by the total number of patient-years on PD (I, II).

#### **4.2.8 Growth in children on peritoneal dialysis**

Height, weight, and head circumference were documented during ward visits (I, II). If accessible, these measures are reported also at birth, as well as at six and three months before PD onset. Height, head circumference, and birth weight values are expressed as mean height standard deviation scores (hSDS), that is, standard deviations of normal Finnish children (Kantero, Tiisala 1971, Sorva et al. 1984). Weight after birth was compared to height and registered as percentiles of Finnish mean values (Sorva et al. 1984). Growth was judged as good, if growth velocity was normal for age and gender, which was defined as either no change or a positive change in hSDS during the study period (I, II, III). Parental target height, also called midparental height, was assessed according to Sorva et al. (Sorva et al. 1989) (II).

Two of the retrospectively analyzed PD patients were excluded from growth analysis, one due to a very short PD period (0.2 years) and the other because of notably deviant growth due to severe congenital heart disease (I).

#### **4.2.9 Neurological development and complications**

##### *4.2.9.1 Risk factors for development*

In our neurological analysis on PD patients with dialysis initiation between 2001 and 2005, risk factors for brain damage and neurological development were determined

as follows: 1) a clear disturbance during the intrauterine period, 2) an Apgar score less than 4 at one minute (ICD-10) or lower than 5 at five minutes (van Schie et al. 2007), 3) intensive care and respirator treatment perinatally or later during infancy before PD initiation, and 4) co-morbidities such as syndromes and brain insults before PD start that are known to affect the child's motor or psychomotor development. Intubation needed for transfer to another hospital was not considered a risk factor for development (III).

#### *4.2.9.2 Neurological examinations and tests*

The same pediatric neurologist examined prospectively all children with PD initiation between 2001 and 2005 when PD was started, every third month until the child turned one year, and every six months thereafter (III). After Tx, the children with neurological abnormalities visited a neurologist at least every year. We collected data about neurological evaluations performed at local hospitals. Children and their development were evaluated also with other methods as follows.

Motor development was investigated with the Alberta Infant Motor scale (AIMS) (Piper et al. 1992) by a physiotherapist (III). AIMS tests were done in all patients at PD start and during ward visits until Tx but not at over 20 months of age. There were two to seven evaluations per patient. AIMS was selected because it is a suitable test battery for infants and small children up until 18 to 20 months of age. Moreover, it was already in use at our center and the physiotherapists were experienced with it.

An occupational therapist conducted a Munich Functional Developmental Diagnostic test (MFED, Münchener funktionelle Entwicklungsdiagnostik test) to assess functional development (Hellbrügge et al. 1978). The test was carried out in 15 children every six months after the age of one until the age of two or at most until Tx (one to four experiments per patient, range 13-20 months). Six patients were transplanted early before any MFED evaluations had been done. Four developmental areas were tested; 1) fine motor skills, 2) perception, 3) social skills, and 4) autonomous activity. Three categories were used in evaluating development; 1) abilities lower than the actual age (<5<sup>th</sup> percentile), 2) normal abilities (between 5<sup>th</sup> and 50<sup>th</sup> percentiles), and 3) good abilities (>50<sup>th</sup> percentile). MFED was chosen because it evaluates functional development in this young age group. MFED was already standardized for Finnish children by Dr. S. Ruoho in 1983 and it was used in the study of Autti-Rämö et al. (Autti-Rämö 1993, Autti-Rämö, Granström 1991b).

Neuropsychological tests were conducted on 16 out of 21 patients (76%) at about five years of age. Other patients were too young for these evaluations. The tests were the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) and parts of the Developmental Neuropsychological Assessment (NEPSY). In some patients, also the Developmental Test of Visual-Motor Integration (VMI) and the Direct Memory Access (DMA) were used. Moreover, we collected data about therapies needed, which were speech or occupational therapy.



#### 4.2.9.3 Brain imaging

To exclude obvious CNS malformations and hemorrhage, brain US was performed in patients under one year of age one month after PD start in patients between 2001 and 2005. In 15 patients, US was done already before PD introduction on clinical grounds. Routine brain MRI (or CT) was performed before renal transplantation (preTx) (N=19 /MRI and N=1 /CT) and two years after transplantation (Tx) (N=12, all MRI). Two more patients were imaged soon after Tx (1 MRI and 1 CT) for clinical reasons. In addition, brain MRI was performed on three newborn children for clinical reasons (III).

#### 4.2.9.4 Hearing tests

Prospectively followed PD patients which were younger than seven months were tested with transient evoked otoacoustic emission (TEOAE) (N=6). A pass criterion of signal-to-noise ratio (SNR) at least 6 dB, at the minimum of three of the five half octave bands was used, with concurrent reproducibility of at least 50%. The same criteria were in use at our neonatal intensive care unit for the auditory screening test. Behavioral observation audiometry (BOA) was done in children at least 7 months of age, every six months during dialysis ((N=19, 1 to 4 studies per patient). The normal audition threshold was 30 dB for 0.5—2 kHz. Most of the children who reached the age of 3 years were checked with conditioned play audiometry although they had already been transplanted (N=12). The normal hearing threshold for 0.5—4 kHz was set at 20 dB. Furthermore, one patient was examined with a brainstem evoked response auditory (BERA) test at his local hospital. One patient died during PD before any hearing test was done.

#### 4.2.9.5 Neurodevelopmental outcome

The neurodevelopmental outcome of prospectively followed PD patients was divided into three groups: 1) normal, 2) minor impairment, and 3) major impairment. The grouping criteria are presented below, see Table 7. Grading of CP was done according to Riegel et al. (Riegel et al. 1995, Hagberg et al. 1975). This classification has earlier been used in a Finnish pediatric neurological study by Lano (Lano 2002). On grade 1, the functional disability is minimal or not notable. On grade 2, the functional limitation is clear but independent walking is possible. On grades 3 and 4, independent walking or movement is not possible.

**Table 7.** Grading criteria for neurological impairment in our PD patients, adapted for the patient material from Lano (2002).

Major impairment	Minor impairment
CP grade 2-4 Hearing defect requiring hearing aid Blindness or moderately lowered vision Epilepsy Hydrocephalus with ventriculoperitoneal shunt Severe cognitive disability	CP grade 1 Specific language impairment Visuomotor dysfunction Attention deficit disorders Mild cognitive disability Dyspraxia Speech or occupational therapy



#### 4.2.10 NPHS1 with muscular dystonia and athetosis

In the retrospective study of six patients with NPHS1 and MDA (IV), the analysis included prenatal and perinatal risk factors, bilirubin and albumin values as a newborn, severe infections during the first six months, acute hemodynamic and neurological insults, and growth data. Neurological data were recorded to define neurological symptoms. Neuroradiology was evaluated. In controls, risk factors, as well as neurological and neuroradiological substance were already collected to a large degree for earlier studies at our center (Qvist et al. 2002, Valanne et al. 2004). Consequently, we collected additional data such as growth, bilirubin, and albumin parameters. In addition, for those six patients with NPHS1+MDA, their *NPHS1* mutation, basic laboratory data, as well as urine organic acids, blood lactate, and viral antibodies, and blood and urine amino acids were documented. Typical renal biopsy findings (classical dilatation of renal tubules) were certified. Their brain imaging was re-evaluated by a pediatric neuroradiologist and a pediatric neurologist. Their EEG reports and auditory examinations were gathered. Neuropsychological test evaluations were collected. For two patients, WPPSI-revised and/or two various visual perception tests had been performed at preschool age and WISC-III or NEPSY at school age.

##### 4.2.10.1 Analysis of mitochondrial DNA

In three patients with MDA, isolation of total DNA from the kidney (2 patients) was done with phenol and chloroform and from the blood (1 patient) with a QI-Aamp Blood Kit (Qiagen, Hilden, Germany). Analysis of the patients' mtDNA was performed with conformation-sensitive gel electrophoresis followed by sequencing, to find mutations and polymorphism. In the other three patients, kidney or blood samples were not accessible.

A novel mtDNA mutation was verified with restriction fragment analysis. A second novel mutation was verified with allele-specific amplification (IV).

#### 4.2.11 Ethical considerations

The study protocol was approved by the Ethics Committee for Pediatrics, Adolescent medicine and Psychiatry at the University of Helsinki, Finland. A written informed consent was obtained from the parents after the purpose and design of this study was thoroughly explained to them.

#### 4.2.12 Statistics

In measures such as growth and test evaluations, patients' ages were corrected according to the expected date of delivery. All statistical analyses were performed with SPSS versions 10.0-17.0 for Windows (SPSS Inc., Chicago, IL, USA). The values are reported as mean  $\pm$  1 standard deviation (SD) or as median and range. Pearson's correlation coefficient was used in simple correlations and Spearman's correlation in non-normally distributed data. Comparisons between values such as growth and

laboratory parameters in normally distributed data were done with the analysis of variance (ANOVA) and Student's T test. The Friedman test was used with Dunn's test for non-normally distributed data when comparing values over time. Associations in non-normally distributed data were analyzed with non-parametric Mann-Whitney U test (non-related samples) and Wilcoxon signed ranks test (related samples). Chi square test with Yates' correction for continuity and Fisher's exact test were used when comparing categorical variables between two groups. Incidences between patient groups were tested with Poisson distribution. Statistical significance was defined as  $p < 0.05$ .

## 5 Results

### 5.1 Nutrition

In the retrospectively analyzed PD patient group, almost 100% of the diet was given by a nasogastric tube (I). In the prospective group, 16 patients (76%) needed a permanent nasogastric tube and one patient a gastrostomy to assure adequate energy intake. Nutritional intake of energy, protein, and some minerals, and vitamins, according to the food records from the prospective group, are presented in Table 8 (II). Energy intake was about 100% of the recommended. Our prospectively studied PD patients received more protein than the safe level for normal growth and development suggests, whereas iron and calcium intakes without supplementation were lower than recommended in the Nordic Nutrition Recommendation (NNR) (Nordic council of Ministers 2004). Phosphate intake was low as planned, as a part of the normal diet in ESRF patients. The intake of selenium, zinc and magnesium was also low compared to NNR (II, unpublished data).

**Table 8.** Nutritional mean intake of energy, protein, minerals, and vitamins in 14 patients, PD initiation between 2001 and 2005 (II [energy and protein intake] and unpublished data [minerals and vitamins]).

Nutritional intake per day			RDA
Energy	kcal/kg	84 ± 22	
% of RDA*		100 ± 26	
Protein	g/kg	2.3 ± 0.6	
% of safety level		181 ± 50	
Iron	mg	4.2 ± 2.2	8
Calcium	mg	428 ± 109	540 - 600
Phosphate	mg	318 ± 122	420 - 470
Potassium	mg	701 ± 285	110 - 1800
Sodium	mg	296 ± 301	
Selenium	µg	6.1 ± 7.5	15 - 25
Zinc	mg	3.7 ± 1.1	5 - 7
Copper	mg	0.13 ± 0.16	
Magnesium	mg	67 ± 26	80 - 120
Vitamin D	µg	7.2 ± 2.5	7.5 - 10
Vitamin C	mg	56 ± 18	20 - 30
Folic acid	µg	64 ± 18	50 - 80
Vitamin B-6	mg	0.5 ± 0.2	0.4 - 0.7
Vitamin B-12	µg	1.2 ± 0.6	0.5 - 0.8
Vitamin A	µg	528 ± 197	300 - 400
Thiamine	mg	0.6 ± 0.2	0.4 - 0.6
Riboflavin	mg	0.8 ± 0.2	0.5 - 0.7
Vitamin E	µg	6.3 ± 3.1	4 - 5

RDA = recommended dietary allowance in healthy children (Nordic Nutrition Recommendation [NNR] 2004)

\* according to NNR 2004 and FAO/WHO/UNU Report 2001  
safety level = minimum of adequate allowance (FAO/WHO/UNU Report 1985)

## 5.2 Medication for uremia

There were no statistical differences in EPO, oral iron, calcium, and alphacalcidol doses between retrospectively analyzed and prospectively studied patients after six months on PD. EPO dose was lower and peroral calcium dose higher in our patients between 2001 and 2005 compared to the retrospective group treated between 1995 and 2000, but the differences were not statistically significant. The retrospectively analyzed patients received significantly more sodium chloride perorally as supplementation; see Table 9 (I, II).

At baseline, in patients with PD onset between 2001 and 2005, alphacalcidol was given daily to 20 patients (95%), while one patient had pulse therapy. At three months, 12 patients (57%) still received daily dosage, seven (33%) had pulse therapy, and for two (10%) the medication was paused due to low iPTH values. At six, nine and twelve months the dosage modes were 13 daily/6 pulse/2 pause, 11 daily/4 pulse/0 pause and 4 daily/5 pulse/0 pause (II). See the iPTH values in these patients in the next chapter.

**Table 9.** Medication information in prospective PD patients at baseline, at 3 and 6 months and in retrospectively analyzed patients at 6 months.

Medication	Dose Study II (n = 21)			Dose Study I (n = 22)	p-value**
	at baseline	3 months	6 months	6 months	
Erythropoietin					
IU per week	1507 ± 899	1304 ± 1013	2143 ± 1088	2545 ± 1301	
IU/kg per week	214 ± 89	162 ± 140	228 ± 121	283 ± 141	ns
Oral iron					
mg per day	21.0 ± 9.4	26.2 ± 11.5	31.4 ± 9.5	33 ± 17	
mg/kg per day	2.8 ± 1.0	3.1 ± 1.2	3.3 ± 0.8	3.6 ± 1.5	ns
Calcium					
mg per day	308 ± 199	368 ± 220	343 ± 283	259 ± 208	
mg/kg per day	45 ± 36	44 ± 26	37 ± 30	26 ± 19	ns
Alphacalcidol					
µg per week	1.2 ± 0.8	2.1 ± 1.8	2.9 ± 2.6	2.0 ± 1.3	
µg/kg per week	0.18 ± 0.13	0.28 ± 0.30	0.34 ± 0.35	0.23 ± 0.15	ns
Sodium chloride*					
mg per day	896 ± 368	887 ± 547	831 ± 594	1499 ± 652	0.005
mg/kg per day	132 ± 66	113 ± 69	87 ± 63	181 ± 118	0.007

\* n = 19 in study I and n = 16 in study II, sodium chloride contains 40% sodium

\*\* comparison at 6 months between study I and II

ns = not significant

## 5.3 Dialysis

Our patients and some outcome measures are presented in Table 10. There was no statistical difference between age, PD duration, number of NPHS1 patients or nephrectomized patients, or in gender distribution between our two patient groups:

1) PD initiation between 1995 and 2000, here referred to as retrospectively analyzed PD patients (I), and 2) PD initiation between 2001 and 2005, here referred to as the prospective group or prospectively followed patients (II). Peritonitis frequency was lower in this prospective group but the difference was not statistically significant.

The retrospectively analyzed PD patient group altogether constituted of 377 dialysis months and the prospective group 284 months. CCPD was the most common dialysis modality when considering treatment time with 66% in the retrospectively analyzed PD patients and 61% in the prospective patients. TPD proportions of dialysis time were 22% and 38% in these groups. Only the retrospectively analyzed PD patients were treated with CAPD (12% of the dialysis time). Five of the retrospectively analyzed PD patients and two of the prospectively followed patients needed HD for some time between PD periods. In the prospective PD patient group, it constituted altogether 3 months (1% of dialysis time). From both groups, three patients were transferred permanently to HD because of difficult peritonitis, ultrafiltration failure, or dialysate leakage (I, II, unpublished data).

**Table 10.** Patient comparison in children under 2 years of age with PD onset between 1995 and 2000 (I) and between 2001 and 2005 (II).

	Study I n=23	Study II n=21	p-value
Patient age at PD onset	0.4 ± 0.3	0.6 ± 0.3	ns
Duration of PD, years; mean ± 1 SD	1.4 ± 0.8	1.1 ± 0.6	ns
Duration of PD, years, range	0.2-3.6	0.5-2.5	
NPHS1 as diagnosis	13 (56%)	15 (71%)	ns
Bilateral nephrectomy	17 (74%)	17 (81%)	ns
Male gender	14 (61%)	12 (57%)	ns
Hospitalization, days per patient-year	124	65	0.03
Peritonitis frequency, per patient-months	1 per 14.5	1 per 17.8	ns
Height SDS at the end of PD	-0.9 ± 1.3 *	-0.86 ± 0.99	ns
Height SDS alteration during PD	+0.48 ± 1.35 *	+0.42 ± 0.86	ns
Mortality, patients (%)	2 (9%)	1 (5%)	

ns = not significant

\* n = 21

## 5.4 Uremia control

### 5.4.1 Laboratory assays

Essential laboratory values are presented in Table 11. There were no statistical differences in creatinine, hemoglobin, calcium or phosphate, PTH, or cholesterol and triglyceride values between retrospectively analyzed and prospectively followed PD patients. PTH and triglyceride values seemed however to be lower in the prospective

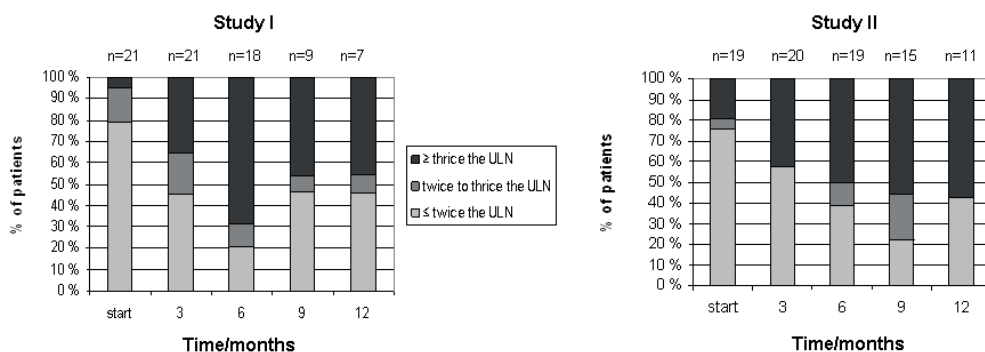
group. BUN values were statistically lower in the prospective group (I, II).

Intact PTH levels were difficult to control in our prospective group. IPTH values in the retrospectively analyzed and in the prospectively followed PD patient groups are shown in Figure 9. In both groups, a clear tendency towards an increase in iPTH during PD can be seen. Differences between these patient groups were not statistically significant.

**Table 11.** Laboratory values at 6 months on PD in children less than two years of age at PD onset between 1995 and 2000 (Study I) and between 2001 and 2005 (Study II).

Laboratory values at 6 months	Study I n=21	Study II n=18	p-value
BUN (mmol/L)	39 ± 11	35 ± 7	0.045
Creatinine (μmol/L)	397 ± 154	473 ± 138	ns
Hemoglobin (g/L)	109 ± 16	112 ± 14	ns
Ionized calcium (mmol/L)	1.25 ± 0.08	1.26 ± 0.07	ns
Phosphate (mmol/L)	1.66 ± 0.61	1.66 ± 0.37	ns
IonCa × Pi (mmol <sup>2</sup> /L <sup>2</sup> )	2.0 ± 0.7	2.1 ± 0.5	ns
Intact PTH (ng/L), median (range)	287 (5-1124)	218 (24-863)	ns
Triglycerides (mmol/L)	3.7 ± 1.5	2.79 ± 1.5	ns
Total cholesterol (mmol/L)	5.6 ± 1.2	5.5 ± 1.5	ns

ns = not significant



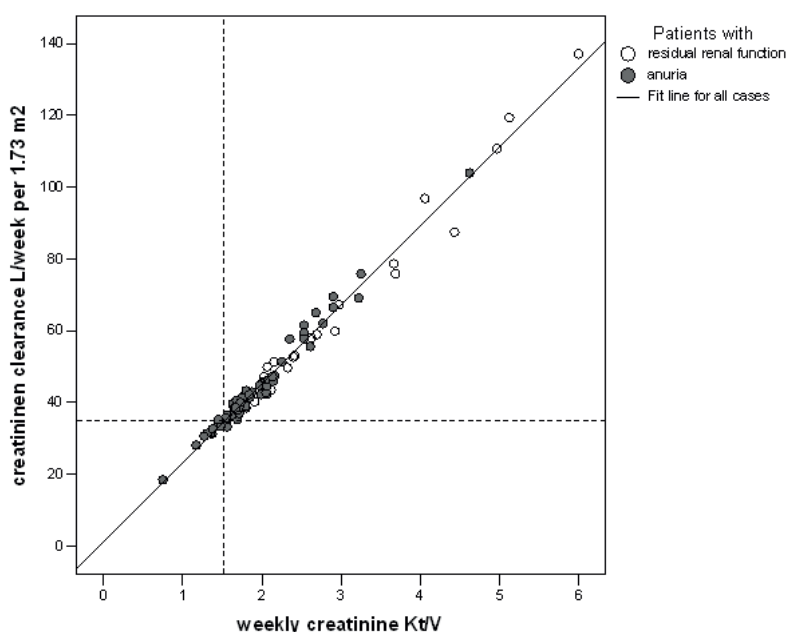
**Figure 9** Percentage of patients with a plasma intact parathyroid hormone level below or over twice and thrice the upper limit of normal (ULN) during dialysis in retrospectively analyzed (I) and prospective PD patients (II). Numbers of patients above the columns.

## 5.4.2 Adequacy of PD

In retrospectively analyzed patients with a modified 24-hour dialysate collection, the adequacy parameters were mean urea Kt/V  $3.2 \pm 1.0$  and mean Crcl  $67 \pm 23$  L/week per  $1.73 \text{ m}^2$  at about nine months on PD (n=15) (I). However, the dialysate collection method was different to our prospectively studied patients (see Methods in chapter 4.2.5.2). In the prospective group, these values were mean urea Kt/V  $3.3 \pm 0.8$  and

mean Crcl  $49 \pm 19$  L/week per  $1.73 \text{ m}^2$  at three months ( $n=21$ ),  $3.5 \pm 0.6$  and  $49 \pm 18$  L/week per  $1.73 \text{ m}^2$  at six months ( $n=18$ ), and  $3.5 \pm 0.8$  and  $49 \pm 30$  L/week/ $1.73 \text{ m}^2$  at nine months ( $n=9$ ), respectively. Patients with RRF showed no significantly higher urea purification compared with anuric patients. The Crcl levels were significantly better in patients with RRF between 2001 and 2005:  $66 \pm 27$  L/week per  $1.73 \text{ m}^2$  compared to  $44 \pm 13$  L/wk per  $1.73 \text{ m}^2$  in anuric patients ( $p<0.001$ ) (II).

The Crcl levels in our prospectively followed PD patients were lower than targeted, in particular in anuric patients. Still, these patients grew well during PD. When our material was analyzed with the method of Ishikura et al. (2003), described in the methods section, the mean weekly creatinine Kt/V was  $2.2 \pm 0.9$  in all patients. In anuric patients it was  $1.9 \pm 0.6$  and  $3.0 \pm 1.2$  in children with RRF. The determined cut-off point; a weekly creatinine Kt/V of 1.52 which corresponded to a Crcl of 60 L/week per  $1.73 \text{ m}^2$  in male adults, was in our patient material equal to a Crcl level of 35 L/week per  $1.73 \text{ m}^2$ , see Figure 10. Thus, if the child is growing and developing well, as low Crcl as 35 L/week per  $1.73 \text{ m}^2$  or over seems to be enough.

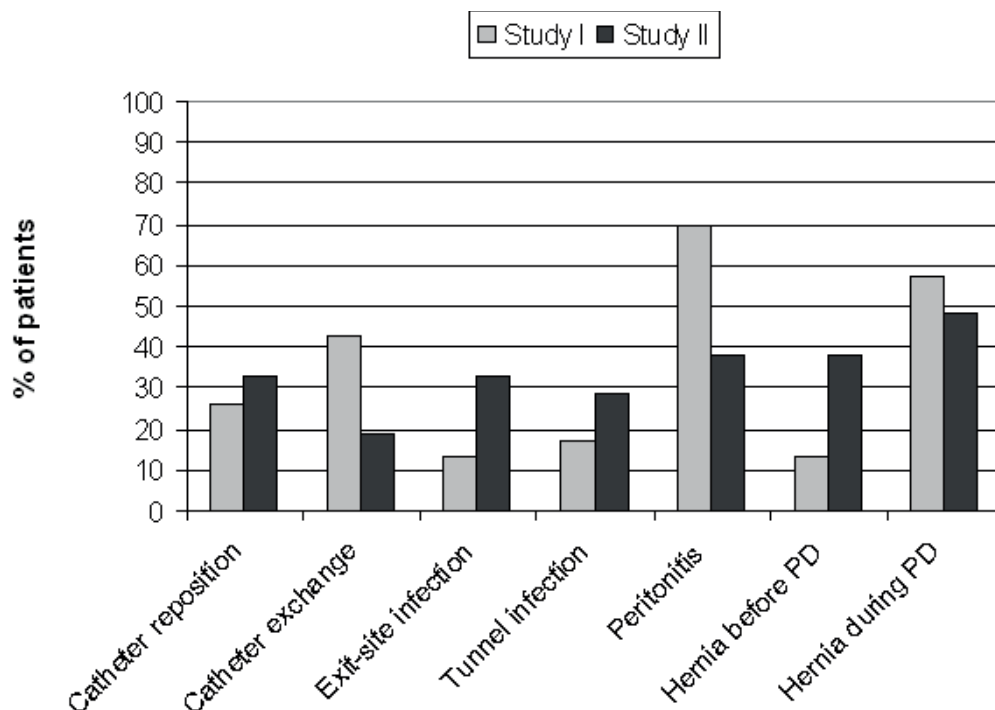


**Figure 10** Creatinine clearances in 21 prospective PD patients less than 2 years of age at onset of PD. Patients with residual renal function are marked with empty circles and anuric patients with filled circles. Fit line represents all measurements. Vertical dashed line shows weekly creatinine Kt/V of 1.52 and horizontal line corresponds to a creatinine clearance value of 35 L/week  $1.73 \text{ m}^2$  (unpublished data).

## 5.5 Complications of PD

### 5.5.1 Catheter-related complications and hernias

The need for surgical intervention for PD catheters, catheter-related infections, peritonitis, and hernias before PD onset and during PD in retrospectively analyzed and prospectively followed PD patients are presented in Figure 11. There were no significant differences in the number of patients having catheter problems or hernias between the groups. There were less catheter exchanges in the prospective PD patient group but the difference was not statistically significant (I, II).



**Figure 11** Catheter repositions and exchanges, tunnel and exit-site infections, peritonitis, and hernia in patients under age 2 at PD initiation (n=23, study I; n=21, study II, unpublished data).

### 5.5.2 Peritonitis

Peritonitis frequency is shown in Tables 10 and 12 (I, II). Peritonitis incidence and causative organisms are presented in Table 12. Prospectively followed PD patients had less peritonitis, but the difference in peritonitis incidence was not statistically significant. However, more prospectively followed PD patients were peritonitis free during the whole PD period compared to retrospectively analyzed patients ( $p=0.036$ ) (I, II and unpublished data). *Staphylococcus aureus* was a frequent organism in the prospective group and 86% of these infections were detected in two patients, whom



both experienced three bouts of peritonitis. In the families of these patients, nasal carriers of *S. aureus* were found (unpublished data).

**Table 12.** Causative organisms of peritonitis in patients under two years of age on PD between 1995 and 2000 (I), and between 2001 and 2005 (II and unpublished data [causative organisms])

	Study I n=23	Study II* n=21	p-value
Total number of peritonitis	26	16	
Incidence; peritonitis per patient month	1 per 14.5	1 per 17.8	ns
Peritonitis-free patients	30 %	62 %	0.036
Gram-positive	37 %	62 %	ns
Coagulase negative Staphylococcus	19 %	6 %	
Staphylococcus aureus	11 %	44 %	0.027
Streptococci	7 %	12 %	
Gram-negative	37 %	13 %	ns
Pseudomonas	15 %	0 %	
Klebsiella	11 %	0 %	
Acinetobacter	4 %	6 %	
Other bacteria	7 %	7 %	
Fungal	4 %	0 %	
Culture negative	22 %	25 %	ns

ns = not significant

\* and unpublished data

### 5.5.3 Intravascular volume status

Blood pressures, plasma ANP-N and BIA values, linear heart dimensions, and LV mass-for-height Z-scores of our prospective PD patients are presented in Table 13. At a stable stage on PD, at 3 months after PD initiation, high plasma ANP-N (n=19) correlated well with both systolic and diastolic hypertension ( $r=0.59$ ,  $p=0.007$  and  $r=0.57$ ,  $p=0.011$ ). LVH (Z-scores, n=19) also correlated well with both systolic and diastolic hypertension ( $r=0.67$ ,  $p=0.002$  and  $r=0.54$ ,  $p=0.017$ ) at three months on PD but not with plasma ANP-N. LVEDD and systolic hypertension (n=15) showed a good correlation at 3 months ( $r=0.62$ ,  $p=0.013$ ). All these preceding correlations of about the same magnitude were also seen at baseline and at 6 months on PD (II). BIA RI correlated only in the beginning of PD with LVEDD (n=12,  $r=0.59$ ,  $p=0.045$ ) (unpublished data). LVEDD diminished significantly between baseline and 3 months ( $p=0.002$ ), as well as between baseline and 6 months ( $p=0.024$ ). The proportion of patients with LVH (counted with Z-scores) decreased during PD. The improvement was statistically significant ( $p=0.016$ ; PD start vs. 3 months and  $p=0.035$ ; PD start vs. 6 months) (II).

Antihypertensive medication was needed in 70% of the retrospectively studied PD patients at some point during PD, while the percentage was only 33% in our prospective group (I, II).

Prospectively followed patients had no clear periods with clinical hypovolemia. Nor did we find data about clinically significant periods of hypovolemia in the patient records of the retrospectively analyzed PD patients (I, II).

In conclusion, LVH decreased in the prospectively followed patients with time during PD. However, many patients still had high blood pressures during PD.

**Table 13.** Blood pressures and patients exceeding the 95th percentile, plasma ANP-N and patients exceeding the value 3 nmol/L, and BIA resistance, resistance index, and reactance. Under the linear heart dimensions and LV mass-for height Z-scores and percentage of patients exceeding the 95th percentile (II and unpublished data [BIA values]).

Volume indicator	PD duration					
	Baseline		3 months		6 months	
	>95th pctl		>95th pctl		>95th pctl	
Systolic BP mmHg <sup>1</sup>	105 ± 18	40%	101 ± 20	29%	104 ± 18	37%
Diastolic BP mmHg <sup>1</sup>	56 ± 10	0%	56 ± 14	14%	55 ± 12	11%
Plasma ANP-N nmol/L <sup>2</sup>	8.9 ± 8.7		2.7 ± 1.2		3.3 ± 1.9	
n and % of patients >3nmol/L	15/18	(83%)	7/19	(37%)	6/17	(35%)
Resistance Ω <sup>3</sup>	768 ± 128		740 ± 104		701 ± 93	
Resistance index m <sup>2</sup> /Ω <sup>3</sup>	5.7 ± 1.0		7.1 ± 0.9		8.3 ± 1.3	
Reactance Ω <sup>3</sup>	53 ± 25		50 ± 11		50 ± 11	
<b>Heart</b>						
<b>dimension</b>	Z-score	>95th pctl	Z-score	>95th pctl	Z-score	>95th pctl
LVEDD*	0.28 ± 1.80	31 %	-1.55 ± 1.55	0 %	-0.83 ± 1.43	6 %
LVESD*	0.21 ± 1.75	19 %	-1.74 ± 1.77	0 %	-1.46 ± 1.97	12 %
SeptD*	2.91 ± 1.80	87 %	3.17 ± 1.72	80 %	3.18 ± 1.17	94 %
LVPWD*	2.34 ± 1.54	56 %	1.79 ± 2.11	60 %	2.21 ± 1.45	59 %
LV mass-for-height**	0.96 ± 1.65	33 %	-0.09 ± 1.94	16 %	-0.07 ± 1.41	18 %

<sup>1</sup> n = 20 at baseline, n = 21 at 3 months, and n = 19 at 6 months, percentiles according to Task Force Report 1996

<sup>2</sup> n = 18 at baseline, n = 19 at 3 months, and n = 17 at 6 months

<sup>3</sup> n = 13 at baseline, n = 13 at 3 months, and n = 12 at 6 months

\* n = 16 at baseline, n = 15 at 3 months, and n = 17 at 6 months, values according to Lester et al. 1987

\*\* n = 21 at baseline, n = 19 at 3 months, and n = 17 at 6 months, values according to Foster et al. 2008

BP = blood pressure, ANP-N = N-terminal natriuretic peptide

LVEDD = left ventricular end-diastolic dimension, LVESD = left ventricular end-systolic dimension

SeptD = interventricular septal dimension at end-diastole, LVPWD = left ventricular posterior wall thickness at end-diastole

## 5.6 Mortality and hospitalization

Between 1995 and 2000 two patients (9%) died during PD (in 31.4 patient- years). One died due to a subarachnoidal hemorrhage, and the second died of congenital heart disease with severe heart insufficiency (I). Between 2001 and 2006, one patient (5%) with NPHS1 and normal neurology died during PD (in 23.7 patient-years) due to pneumonia after an anesthesia complication. None of these prospectively followed patients died after PD during the neurological follow-up (II). See Table 10.

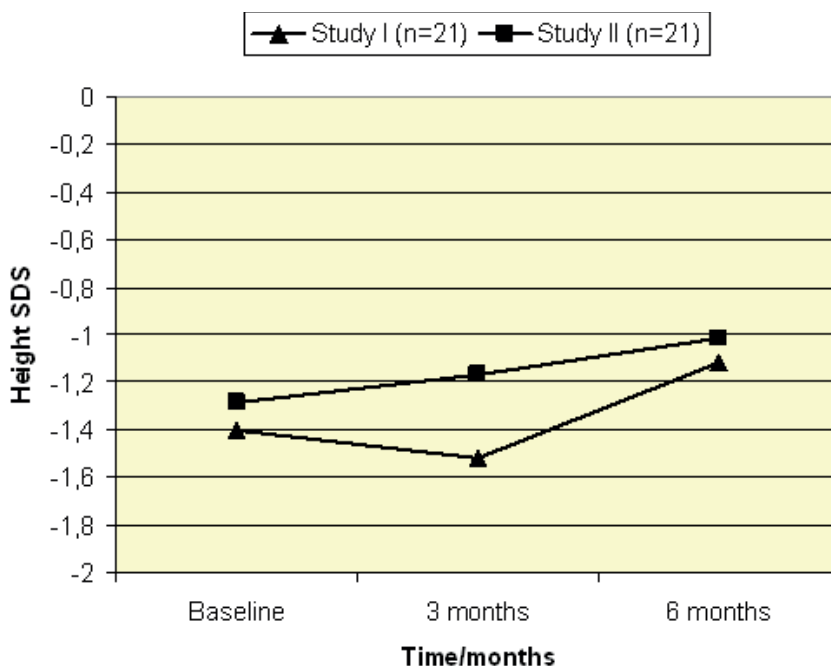
Hospitalization is shown in Table 10. Hospitalization time was significantly shorter in patients with PD onset between 2001 and 2005 (I, II).

## 5.7 Growth

There were no significant differences in hSDSs in the beginning of PD, at three or six months on PD in the retrospectively analyzed PD patients or in those prospectively followed, nor between these two groups. Our prospective PD patients were significantly taller at the end of dialysis compared to the PD onset ( $p=0.036$ ); the mean hSDS at baseline was  $-1.3 \pm 1.2$  and  $-0.9 \pm 1.0$  at the end of PD. Catch-up growth was detected in 64% and 57% of the patients in these groups (I, II).

In the prospectively followed patients, their height was also compared with their parental target height. Although these children grew well, they were significantly shorter during dialysis and at the end of PD at a mean age of 1.71 years compared with their parental target height predictions ( $P < 0.01$  at baseline, 6 months on PD, and at the end of PD) (II).

The mean head circumference (hcf) SDS ( $n=14$ ) in our prospective PD patient group was  $-1.0 \pm 1.1$  at the beginning of PD and  $-1.2 \pm 1.1$  at nine months. The difference was not statistically significant. Three patients (14%) had microcephaly (hcfSDS lower than  $-2.0$ ) at baseline and at the end of PD, two of them at both time points. In all, microcephaly was seen in five patients at some point during PD. One of them had NPHS1, one had multicystic kidneys, and the others had some co-morbidity (III).



**Figure 12** Growth in patients less than two years of age at dialysis onset treated on PD for at least 6 months between 1995 and 2000 (study I), and 2001 and 2005 (study II, III). Mean height SDS at baseline, and at three and six months on PD.

## 5.8 Neurological development (III)

### 5.8.1 Risk factors for development and birth

Altogether eleven patients (52%) in our prospectively followed group were found to have one or more risk factors for their neurodevelopment, detected before PD onset. Six children had perinatal asphyxia (29%) and others had neonatal risk factors. Six children (29%) had co-morbidities. See Table 14.

More than half of our patients (57%) were born prematurely. Their mean hSDS at birth was  $-0.7 \pm 1.5$ , mean birth weight was  $2549 \pm 596$ g, and five (24%) were small for gestational age (SGA). Head circumference SDS was less than -2.0 in three of fifteen measured patients (20%). Two of them also had short stature, so they were small but balanced. Prematurity, SGA, or small head circumference were not considered as risk factors for their neurological development but they were added in statistical analyses when examining explanatory factors for neurological abnormalities. See details in Table 14.

**Table 14.** Demographics and risk factors for neurological development in prospectively followed PD patients less than two years of age at PD initiation. At the end of this study, patients no. 1-6 had normal neurodevelopment; patients **no. 7-15** had minor impairment; and patients **no. 16-21** major impairment (III).

Patient no.	Sex	Diagnosis leading to ESRF	Gestational age /weeks	Weight at birth /gr	Neonatal risk factor	Other risk factors	Risk factor or not
1	M	NPHS1	36	3450	-	-	no
2	F	NPHS1	38	2320/SGA	-	-	no
3	M	NPHS1	38	2710	perinatal asphyxia	-	yes
4	M	NPHS1	37	2650	perinatal asphyxia	-	yes
5	M	Hypoxaemic renal injury	38	3240	severe hypoglycemia	insulinoma and severe hypotension at 2 wks	yes
6	M	NPHS1	36	2355	-	-	no
7	M	NPHS1	37	3080	-	-	no
8	F	NPHS1	34	1810/SGA	-	-	no
9	F	NPHS1	35	2280	-	-	no
10	F	NPHS1	35	1952/SGA	-	-	no
11	M	NPHS1	31	1445/SGA	-	-	no
12	M	NPHS1	37	2930	-	-	no
13	M	NPHS1	30	1720	-	alcohol exposure in utero	yes
14	M	Urethral valve	38	3510	-	-	no
15	F	ARPKD	34	3395	perinatal asphyxia	hypoplastic lungs	yes
16	M	NPHS1	31	2245	severe RDS, intensive care	-	yes
17	F	NPHS1	34	2100	perinatal asphyxia	-	yes
18	M	NPHS1	35	2250	perinatal asphyxia	sinus sagittalis thrombosis	yes
19	F	Fibromuscular dysplasia	37	2260/SGA	-	underlying syndrome	yes
20	F	Multicystic kidney dysplasia	35	2890	perinatal asphyxia	-	yes
21	F	Townes-Brocks syndrome	39	2940	-	underlying syndrome	yes

NPHS1 = congenital nephrotic syndrome of the Finnish type  
ARPKD = autosomal recessive polycystic kidney disease

SGA = small for gestational age  
RDS = respiratory distress syndrome

### 5.8.2 Brain imaging

In three patients with perinatal asphyxia, brain infarcts were detected soon after birth by MRI. One of them also had a thrombosis of sinus sagittalis. In the patient

with insulinoma and severe hypotension at two weeks, MRI showed an occipital infarct on the right after this episode. In three other patients, US showed some other abnormality as newborn: germinal matrix cysts, lenticulostriatal vasculopathy, and ventriculomegaly in one patient each. These minor abnormalities were later normalized in the first two patients. The patient with ventriculomegaly and fibromuscular dysplasia is discussed below.

In the brain MRIs/CTs (n=19/1), examined during PD as a part of pretransplantation studies, in addition to those four already earlier detected infarcts, one patient with NPHS1 had periventricular leukomalacia (PVL). Also the patient with Townes-Brocks syndrome had developed ventriculomegaly and needed a ventriculo-peritoneal shunt after renal Tx. Four NPHS1 patients had widened cerebrospinal fluid (CSF) spaces in their brain MRI during PD but the findings were normalized after Tx. These temporary changes of CSF spaces have also earlier been documented in NPHS1 patients during PD (Valanne et al. 2004).

After the kidney Tx, eight of fourteen patients with either MRI or CT evaluations (57%) had normal findings. The patient with fibromuscular dysplasia and ventriculomegaly, detected as a newborn, suffered from transient right hemiparesis after transplantation due to a stenotic middle cerebral artery. This finding was later confirmed with brain angiography. Yet another NPHS1 patient with previously normal findings was found to have mild periventricular ischemia after Tx. (Table 2 in Study III, Laakkonen et al. in press).

In summary, four patients (19%) had brain infarcts, detected before PD initiation, and three more patients (14%) had PVL or other ischemic lesions. PVL was due to perinatal asphyxia. New findings were seen after Tx in only two patients. In the first one, born preterm, no clear reason was found, but the ischemia was periventricular referring to a perinatal etiology. In the second one, fibromuscular dysplasia caused the ischemic lesions. Children with risk factors for their neurological development had abnormal brain images more often at some time point during the study ( $p=0.002$ ). The number of abnormal brain images at any time-point of this study, is shown in Table 15.

### **5.8.3 Hearing**

Two children with NPHS1 had sensorineural hearing loss. One was diagnosed in the beginning of PD and the other after renal Tx. The patient with Townes-Brocks syndrome had a combined hearing loss (sensorineural and conductive defect) discovered after Tx. Townes-Brocks syndrome has been shown to cause hearing loss in about 62% of the patients (range 11 to 100%) (Powell, Michaelis 1999). All these three patients needed a hearing aid. In three other patients, normal hearing could not be proven because no hearing test had yet been done after Tx. In 14 of 20 examined patients (70%) the hearing was normal. See Table 15.

### **5.8.4 Neurological follow-up**

The results of the neurological evaluations during PD are shown in Table 15. All patients in our prospective group, examined with AIMS tests, were below or near

the mean for the normal population in their neuromotor development during PD in infancy. The normal level was reached over time faster in patients with no risk factors for development. At one year of age, almost all patients without risk factors for development reached the normal level while almost all patient with risk factors developed slowly and stayed below the normal level of neuromotor development according to AIMS tests (Figures 1 a and b in study III, Laakkonen et al. in press). Seventeen children (81%) received physiotherapy during PD. In children over one year of age on PD functional development was good according to MFED tests performed by the occupational therapist. For only one of the patients of 15 studied, the functional developmental age was abnormal during PD; this child had a co-morbidity (pat. no. 21, see Table 14).

Three patients (14%) developed CP. All three were premature, had perinatal asphyxia, and brain infarcts or PVL explaining their handicap. Two of these patients had also cognitive disability, in one of them only a mild one. Twelve other patients (57%) had some other neurodevelopmental abnormalities. Two patients with co-morbidity belonged to this group. These disabilities and their frequencies are shown in Table 15. Neurological abnormalities in these patients could not explain the preterm birth, low birth weight (SGA), birth head circumference less than -2 SD, low Apgar scores, need for special care as newborn, co-morbidity or nephrectomy.

### **5.8.5 Neurodevelopmental outcome**

At the end of this study, six of 21 (29%) prospectively followed patients were evaluated to have normal neurodevelopment. Neurodevelopmental impairment, including all neurological abnormalities and hearing defects at the end of our study, was detected altogether in 15 patients. Six patients (29%) had a major impairment and nine (43%) a minor impairment (Table 15). The three different groups of neurological outcomes (normal development/minor impairment/major impairment) are shown in Table 14. The neurological problems in these patients did not worsen during PD and all children tolerated PD well. The patients with risk factors for development had more often major impairment compared to other patients ( $p=0.004$ ).

Every one of the school aged children ( $n=15$ ) attended full-time school. Two of them (13%) needed part-time and five (33%), all with major impairment, needed full-time special education.

**Table 15.** The proportion of children with abnormal findings in neurological evaluations (AIMS, MFED, and neuropsychological tests), brain imaging, hearing and neurodevelopmental outcome in prospectively followed PD patients under two years of age at PD onset (III). AIMS, Alberta Infant Motor Scale. MFED, Munich Functional Developmental Diagnostic test.

<b>Patients n (%)</b>	
Total n = 21	
<b>Neurological evaluations</b>	
Last AIMS evaluation	
under the 5th percentile	11 (52%)
MFED under the 5th percentile (n=15)	
in 2 of 4 evaluated areas	1 (5%)
Neuropsychological tests (n=16)	
cognitive disability	3 (14%)
verbal learning disability <sup>1</sup>	2 (10%)
specific language impairment	2 (10%)
attention deficit disorders	2 (10%)
visuomotor dysfunction	2 (10%)
dyspraxia	2 (10%)
<b>Brain imaging</b>	
Deviant study	12 (57%)
Brain US deviant	6 (29%)
Brain MRI/CT deviant	12 (57%)
<b>Hearing</b>	
SNHL	2 (10%)
Combined hearing loss	1 (5%)
<b>Neurodevelopmental outcome</b>	
Major impairment	6 (29%) <sup>2</sup>
CP	2 (10%)
other	4 (19%)
Minor impairment	9 (43%) <sup>3</sup>
CP	1 (5%)
other	8 (38%)

SNHL, sensorineural hearing loss

CP, cerebral palsy

<sup>1</sup> in children with a hearing defect

<sup>2</sup> all had a risk factor for development

<sup>3</sup> two patients had a risk factor for development

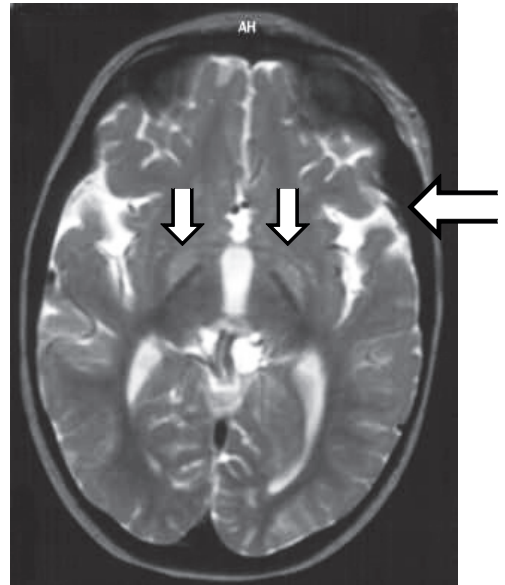
## 5.9 NPHS1 with muscular dystonia and athetosis (IV)

NPHS1 with muscular dystonia and athetosis (MDA) is a new syndrome not described earlier in the literature. All these six patients had a typical renal histology of CNF, severe early proteinuria with hypoalbuminemia and normal glomerular filtration rate (GFR). Four patients were Fin-major homozygotes and one was Fin-major and Fin-minor heterozygote. In one patient, the NPHS1 mutation analysis was not done. The patients' neurological syndrome MDA onset took place between the neonatal period and 8 months of age. It was diagnosed before 11 months of age in all patients and worsened during stress situations like infections. EEG did not reveal epileptic discharges in any of these patients.

These patients were significantly shorter than the controls. At the age of nine months their mean hSDS was  $-3.1 \pm 1.1$  compared to  $-1.4 \pm 1.4$  in the controls ( $p=0.01$ ).

Brain MRI ( $n=4$ ) showed increased signal activity in the globus pallidus area in all imaged NPHS1+MDA patients (Figure 13). In MRI and CT, watershed area infarcts were seen in three patients. In the number of patients with a brain infarction, the difference between NPHS1+MDA patients and controls was not significant. Widened cerebrospinal fluid (CSF) spaces were seen in four patients with NPHS1+MDA (67%).

A hearing defect was present in four of the five tested patients; three of which had severe SNHL and one mild SNHL. In the two remaining patients, the hearing could not be proven totally normal because of an early death. There were statistical differences in the frequency of hearing deficit between NPHS1+MDA patients and controls ( $p=0.017$ ).



**Figure 13** Brain MRI in a 21 months old patient with NPHS1 with MDA (axial T2-weighted image), indicating increased signal activity in the globus pallidus area. Small vertical arrows show these areas. Mild widening of the CSF space is seen in Sylvian fissures, horizontal arrow showing the right side.

### 5.9.1 Explaining factors for the neurological syndrome

External factors like neonatal events and complications, prevalence of brain infarcts, or septic infections during the first six months did not explain the neurological abnormality of the NPHS1+MDA patients, who needed more frequent special care as a newborn compared to the controls.

An emphasis was given to the detection of neonatal bilirubin values and bilirubin-albumin ratio because both brain MRI/CT findings (increased signal intensity in globus pallidus area) and the neurological syndrome strongly resembled bilirubin



encephalopathy. Eighty per cent of the NPHS1+MDA patients and 59% of the controls had a serum bilirubin-albumin molar ratio higher than 0.63, which has been thought to be a cut-off point of inducing a hearing defect. The limit for neurotoxicity in serum bilirubin-albumin molar ratio is 0.8 and 50% of NPHS1+MDA patients and 15% of controls exceeded it. However, the controls with molar ratios as high as in NPHS1+MDA patients, did not even have a hearing defect. Thus, this condition could not be explained by plain chronic bilirubin encephalopathy although the low serum albumin level in newborn NPHS1 patients could be a risk factor.

Brain infarcts were equally common in both patients groups, in 50% of NPHS1+MDA patients and in 59% of controls. Control patients had more severe infarctions compared to NPHS1+MDA patients. Therefore, MDA can not be explained by brain infarcts.

Plasma and urine aminoacids, urinary organic acids, blood and liquor lactate, blood pyruvate and ammonium levels, as well as serum creatinine kinase measured in some NPHS1+MDA patients did not reveal any metabolic disease that could have explained the MDA symptoms. The EEGs, taken because of convulsion-like seizures, showed no epileptic activity in any of these patients. For one patient's muscle biopsy, electron microscopy and mitochondrial enzyme tests were normal. Also an electroneuromyography (ENMG) study in one patient was normal.

MtDNA was analyzed in three patients. No known mitochondrial mutation that could explain MDA was found in these patients. Together 24 nucleotide position aberrations were found. Twenty-two of them were common polymorphisms or found in databases. Two different, new mutations were found and their implication could not be explained.

### **5.9.2 Outcome of patients with muscular dystonia and athetosis**

Four of the NPHS1 patients with MDA (67%) died early. Two of them died during dialysis between 1 and 2 years, one due to peritonitis and another with myocardial hypertrophy and its' consequences. Two patients died after renal Tx, one with pneumonia at the age of two years and another because of multiorgan failure following Tx at the age of three years. Among the other NPHS1 patients born during the same time period as these NPHS1+MDA patients, only six (9%) had died.

Two NPHS1 patients which survived had functioning renal grafts, severe dystonia, and need for wheelchair and hearing aid at the end of the study.



## 6 Discussion

Nowadays even the youngest patients, infants, are treated actively with peritoneal dialysis. Therefore, we wanted to evaluate the adequacy of dialysis, metabolic and fluid balance, complications, growth, and neurological development in this patient group, and on that basis construct suitable medication, nutrition, peritoneal dialysis prescription, and adequate, but not too exhausting, follow-up programs for these patients. We first evaluated the preceding facts in a retrospective PD population, treated in our center between 1995 and 2000 and started a prospective study of PD patients of similar age in 2001, treating 21 patients with this protocol. Neurological follow-up was one part of this prospective study. We also described a small NPHS1 patient population with a severe neurological syndrome in order to establish an explanation for their symptoms.

### 6.1 Supportive treatment

#### 6.1.1 Nutrition (II)

Nutrition provided enough energy for the age group in our prospectively followed patients. The mean amount of energy was 100% of the RDA in healthy children of this age. Comparatively, the amount of energy in the study by Hölttä et al. (2000b) in children under five years of age (between 1995 and 2000) was 109% of the RDA at baseline, which is almost the same as in our patients treated between 2001 and 2005. Catch-up growth was observed in most patients in both of these studies. Our patients' protein intake was higher than recommended in the newest Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines, with a mean intake of  $2.3 \pm 0.6$  g/kg per day. In contrast, the recent recommendations are 1.5–1.8 g/kg per day for children under one year and 1.3 g/kg per day for children between one and three years (KDOQI Work Group 2009). The same amount of proteins has been provided to children less than five years of age in the above mentioned study by Hölttä et al. (Hölttä et al. 2000b). High protein intake increases the need of phosphate binders which are mainly calcium-based. When we counted together dietary calcium, which was fairly low, and calcium from phosphate binders at three months on PD, it still remained under the upper limit of the KDOQI recommendation of 1000 mg/day for children between one and three years. However, the KDOQI guide for children between six and twelve months is not more than 540 mg/day, which we exceeded (KDOQI Work Group 2009). In fact, 40% of our patients had an ion-Ca  $\times$  Pi higher than recommended. A high calcium phosphorus product is a risk for vascular calcification and should be avoided (Klaus et al. 2006). Phosphate binders that contain a high amount of calcium increase the calcium load of patients. New calcium free phosphate binders are, however, not yet officially registered for small children. Most of our infants with ESRF have protein malnutrition before PD due to severe protein losses through their kidneys before nephrectomy as they are nephrotic. Although these infants need

higher protein substitution at the commencement of PD, their protein intake should probably be reduced after a few months on PD, and at the age of one year, to reduce the risks for vascular calcification.

### **6.1.2 Medication (II)**

Daily dosing of vitamin D was used between 2001 and 2005. Our aim was to provide a more physiological calcium-phosphate-PTH balance for bone mineralization compared to pulse therapy. Pulse therapy of vitamin D has been claimed to reduce bone formation and reduce linear growth in children with secondary hyperparathyroidism. PTH levels were lower in children with adynamic bone disease during intermittent calcitriol therapy compared to children without adynamic bone disease (Goodman et al. 1994, Kuizon et al. 1998). The European Paediatric Dialysis Working Group recommended PTH values of two to three times the upper limit of normal during PD (Klaus et al. 2006). We aimed to reach normal or two-fold the upper normal limit concerning iPTH. These levels were difficult to control in our prospective patient group and alphacalcidol doses had to be readjusted repeatedly in order to keep iPTH at target levels. Moreover, about 50% of our patients had to be switched to pulse therapy. In Finland, it has been shown in children with CKD and secondary parathyroidism (mean age 5.6 years) by Ala-Houhala et al. (Ala-Houhala et al. 1995) that alphacalcidol pulses normalize and stabilize PTH levels well in the predialysis period. During follow-up, vitamin D metabolite concentrations were normal, growth was low normal, and bone mineral density did not decrease. This knowledge formed the basis for our procedure when changing our patients to the pulse therapy when PTH rose rapidly or was very high. A more recent study in Finland also showed, in a small group of infant patients, that growth was better with pulse therapy compared to daily dosing of vitamin D (Saarinen et al. 2007). Vitamin D analogs or calcimimetics were not used in our patients due to scarce experience in this age group. Serum FGF23 levels have been studied by Kazama et al. (Kazama et al. 2005) and Nakanishi et al. (Nakanishi et al. 2005) in adult HD patients, whose iPTH levels were greater than 300 ng/L. They all concluded that serum FGF23 level was a good indicator in prognosticating the response to vitamin D therapy and a valuable factor in predicting the course of secondary hyperparathyroidism in patients with chronic dialysis (Kazama et al. 2005, Nakanishi et al. 2005). In the future, follow-up of the circulating concentration of FGF23 blood level may provide a good tool for the evaluation of calcium-phosphorus-vitamin D balance in secondary hyperparathyroidism in pediatric PD patients, especially together with iPTH assays.

Mean dietary iron was only around 50% of the daily recommendation in our prospectively followed PD patients. Moreover, uremia and calcium containing phosphate binders worsen absorption of iron from the diet. Thus, our patients received iron and EPO supplementation to attain normal red cell formation and normal blood hemoglobin, which were reached.

## 6.2 Peritoneal dialysis

### 6.2.1 Uremia control (I, II)

Metabolic control was good in our patients between 1995 and 2005. There were no differences in hemoglobin, creatinine, calcium, phosphate, iPTH, cholesterol, or triglyceride values between patients treated during 1995–2000 and 2001–2005. BUN values were statistically lower in patients treated in 2001–2005. Low BUN values, despite high protein intake, were probably due to high levels of urea purification reached in our patients; see below. The difficulty in controlling iPTH values, even in prospectively studied patients, is discussed above. Favorable metabolic control is also seen in other studies in older children on PD (Schaefer et al. 1999a, Chadha et al. 2001).

The weekly Kt/V levels for urea, describing the clearance of small-sized molecules, were good during dialysis between 2001 and 2005 (mean at six months  $3.5 \pm 0.6$ ), as well as in the retrospectively studied patients between 1995 and 2000 (mean at nine months  $3.2 \pm 1.0$ ). Our target of 3.0 in the prospective patients was well reached. Differences in Kt/V for urea were not significant between anuric and RRF patients. In studies on children of all ages, a mean Kt/V urea of 2.0 to 3.45 has been reported (Schaefer et al. 1999a, Chadha et al. 2001, van der Voort et al. 2000): our results in infants are comparable to them. Acceptable clearance of mid-sized molecules was harder to achieve in our prospectively studied PD patients. In the retrospective patient group, the dialysate collection was modified and the Crcl values raised by 15% (Hölttä et al. 2000b). Thus, the mean Crcl of  $67 \pm 23$  L/week per  $1.73 \text{ m}^2$  at nine months on PD is not comparable to the Crcl of  $49 \pm 18$  L/week per  $1.73 \text{ m}^2$  in prospectively studied patients. Especially in nephrectomized children, the Crcl values between 2001 and 2005 remained far behind our target of 65 L/week per  $1.73 \text{ m}^2$  ( $44 \pm 13$  L/wk per  $1.73 \text{ m}^2$  in anuric patients vs.  $66 \pm 27$  L/week per  $1.73 \text{ m}^2$  in children with RRF). Patients with RRF had significantly better Crcl. Schaefer et al. reported a mean Crcl of  $53 \pm 24$  L/week per  $1.73 \text{ m}^2$  in 51 infants on PD (Schaefer et al. 1999a), whilst Chadha et al. (2001) observed a mean Crcl of  $70 \pm 18$  L/week per  $1.73 \text{ m}^2$  in 24 children (50% anuric). They also noted that anuric children had a notably lower mean Crcl ( $59$  L/week per  $1.73 \text{ m}^2$ ) compared to children with RRF ( $81$  L/week per  $1.73 \text{ m}^2$ ) (Chadha et al. 2001). However, Crcl is related to BSA that is proportionally large in infants compared to older children and adults. This fact may partly explain lower Crcl values, particularly in infants. Another method to evaluate creatinine purification is weekly Kt/V for creatinine which correlates the clearance with total body water, not with BSA (Ishikura et al. 2003). In our patients, the mean Crcl was  $49 \pm 20$  L/week per  $1.73 \text{ m}^2$  and the mean creatinine Kt/V  $2.19 \pm 0.91$ , which being higher than 1.52 correspond to Crcl of 60 in an adult male. A weekly Kt/V for creatinine of 1.52 is equivalent to a Crcl of 35 L/week per  $1.73 \text{ m}^2$ . Thus, we recommend Crcl values of over 40 L/week per  $1.73 \text{ m}^2$  as acceptable in infants if their growth is good. However, the use of creatinine Kt/V needs to be tested in a larger population of children in the future.

## 6.3 Complications of peritoneal dialysis

### 6.3.1 Catheter-related problems, peritonitis and hernias (I, II)

Catheter-related technical problems were common. Repositions of catheters were equally common during 1995–2000 as during 2001–2005, being necessary in roughly 30% of the patients. Catheter exchanges were less common in the prospectively followed patients, in only 19% versus 43% in the retrospective study but this difference was not statistically significant. Rees reported catheter exchanges in 60% of their infants on PD, three times as frequently as in the patients treated in our clinic during 2001–2005 (Rees 2002). Other studies report catheter exchanges in about 20% of children and adolescents (NAPRTCS 2007 Annual Report 2008, Macchini et al. 2006, Neu et al. 1995, Aksu et al. 2007), similar to our results in infants between 1995 and 2000. It is probable that there will be less malposition problems with the new Flex-Neck® infant catheter, which has a shorter intraperitoneal part. So far, there are no publications about these newer catheters in children.

The annualized peritonitis rate in our patients was 0.83 between 1995 and 2000 and 0.67 between 2001 and 2005. Earlier reports on Finnish patients indicate that the peritonitis rate in children under five years of age had been 1.64 between 1986 and 1994 and 1.28 between 1995 and 1997 (Hölttä et al. 1997, Hölttä et al. 2000b). The NAPRTCS report of 2007 showed a rate of 0.86 in children under two years of age (NAPRTCS 2007 Annual Report 2008) and a recent report of Wedekin et al. (2010) showed a peritonitis rate of 0.85 in infants. Most reports show a higher peritonitis rate in younger children compared to the older ones (Neu et al. 2002, NAPRTCS 2007 Annual Report 2008, Hölttä et al. 2000b, Honda et al. 1996, Hoshii et al. 2006). For example, Boehm et al. (Boehm et al. 2005) reported in 2005 a peritonitis rate of 1.22 in children under two and 0.52 in older children. They also compared the peritonitis rate between anuric children (1.41) and children with RRF (0.61) and stated that anuria is a risk factor for peritonitis. Anuric patients also have day-time exchanges, and thus have more connections, which increase the possibility of infections. However, in our study between 1995 and 2000, the anuric patients had a peritonitis rate of 0.81, and the children with RRF a higher rate of 0.85, so there was no significant difference. Our results have steadily and significantly improved with time, with the peritonitis rate in infants treated between 2001 and 2005 being comparable to that in older children. Careful training of the families, good hygiene, and developed PD connectors probably form an important part of this development. Anuria did not increase the peritonitis rate.

Hernias, mostly inguinal, were common in our patients: 57% of our retrospectively analyzed PD patients needed a hernia operation during PD, similar to the 48% seen for the prospectively followed patients. Hernias were operated prior to PD initiation in 15% and 38% of the patients, respectively. These differences were not statistically significant. Hernias were reported previously in 29% of children under five years of age in Finland (Hölttä et al. 1997). Thus, we comparatively saw more hernias in the youngest patients. The ESRF patients, and especially the NPHS1 patients, have hypoproteinemia prior to PD, and their tissues might be more vulnerable to hernias. Despite this fact, hernias were not seen more frequently in NPHS1 patients in our patient populations.

Furthermore, hernias were frequently seen also elsewhere in PD patients, especially in small children. Hernias were observed in 23 to 40% of children during PD in some earlier studies and also in a more recent one (von Lilien et al. 1987, Khoury et al. 1991, Verrina et al. 1992, Jander et al. 2006). It seems that infants are more prone to hernias and thus the need for a preemptive hernia operation at catheter insertion should be considered in the youngest patients.

IPP should be monitored during PD to detect excessive IPP values in order to avoid hernias and leakages.

### **6.3.2 Intravascular volume status (II)**

Hypertension was detected in 57% of our prospectively followed patients at some point during PD, all of which were anuric. In our retrospective patient group, the frequency of high BP could not be defined, however a previous study in our center reported high BP in 73% of patients less than five years of age on PD (Hölttä et al. 2001). Although hypertension was less common between 2001 and 2005, it still remained a problem during PD. Antihypertensive medication was used in 70% of patients at some point during PD between 1995 and 2000, but in only 33% of our prospective PD patients. Plasma ANP-N correlated moderately with hypertension, indicating hypervolemia in patients with high BP. Due to the lack of normal values for BIA measures for infants and small children, BIA was not very helpful in estimating intravascular volume status. Nor did it correlate with high blood pressure. It is possible that BIA RI was not the right measure for the correlation analyses, but we found no better measure from the literature. Nevertheless, strict intervention for high blood pressure is important in anuric children. If the child is hypervolemic, ultrafiltration should be increased. If no hypervolemia is present, antihypertensive medication should be started. On the other hand, severe hypovolemia is a risk during PD, especially if the child has an infection or is vomiting, and hypovolemia may cause brain damage (Lapeyraque et al. 2003). Thus, it is probably safer to permit mild hypervolemia in infants on PD.

At the commencement of PD, a total of 86% of the prospectively studied PD patients had LVH when using LVMI and the 95th percentile as a cut-off point for LVH, compared with 60% in patients less than five years of age treated between 1995 and 1999 at our center (Hölttä et al. 2001). Surprisingly, according to one of the newest studies on young PD and renal TX patients, in 57% of the healthy, school-aged control children, the LVMI exceeded the 95th percentile (Ten Harkel et al. 2009). This raises a question about the reliability of LVMI in determining LVH. Quantification of left ventricular mass (LVM) and determining the limit for LVH are controversial (Foppa et al. 2005). Small children are a particular challenge in the use of LVMI, which is proportioned to height, thus increasing more rapidly in short children. Thus, one may ask if the often used LVMI represents the complex relationship between LVM and body size during the whole childhood while body proportions change substantially, especially during infancy (Foster et al. 2008). Foster et al. (2008) have focused on LVH definition problems. They developed LV mass-for-height reference centile curves and compared them with the LVMI method in evaluating the proportion of patients with LVH in children with ESRF.



They concluded that their new method normalizes LVM for body size, and thereby especially in small children, less than 140 cm tall, gives a more precise estimate of LVH. Thus, we used Foster's et al. LV mass-for-height and the 95th percentile as a cut-off point for LVH, and LVH was seen in only 33% of our patients treated between 2001 and 2005 at baseline. The difference between frequencies of LVH calculated with the two different methods; 86% and 33% of the patients, is noteworthy. The proportion of patients with LVH, calculated with LV mass-for-height, decreased with time during PD, which also indicates successful management of intravascular volume status.

## 6.4 Mortality and hospitalization (I, II)

Mortality was low in our patients, 9% between 1995 and 2000, and 5% between 2001 and 2005. Rees reported four deaths in 20 infants (20%) during PD (Rees 2002) and a 10% mortality was observed in the study by Shroff et al. Relative risk for death was 2.7 times higher in children under five at the start of dialysis compared to older children (Shroff et al. 2006). Co-morbidity has been shown to be a significant risk factor for higher mortality in children during ESRF and PD (Shroff et al. 2006, Kari et al. 2000).

Hospitalization times have decreased steadily during the last years in small PD patients in Finland. In the early 1990's, the patients under five years of age stayed at hospital for 150 days per patient-year. Later in the 1990's, the number was 95 days per patient-year (Hölttä et al. 2000b). Training of the families developed in the 1990's and thus many children could be treated with PD at home. In our retrospectively analyzed patients, the hospitalization time was again higher; 124 days per patient-year. However, there were three children who had to spend their whole dialysis period in hospital; one due to a severe heart disease, the other two for social reasons. When we excluded these three patients, the hospitalization time is reduced to 93 days per patient-year, which is comparable to the report by Hölttä et al. from about the same time period. Hospitalization time in our prospectively followed PD patients was significantly lower, only 65 days per patient-year. Most of these hospital days consisted of routine PD controls, with complications being the second largest group. The reduction of complications, effective training of the families and short, one to three days long routine ward visits have reduced the time spent in hospital. While this improves the quality of life for both the child and his or her family, one may question whether the ward visits are too stressful for the patient with all the examinations, including pre-transplantation studies, spanning only a few days. In an earlier report by Becker et al., the hospitalization times were only 0 to 30 days per year (mean 14.5 days) in children under five years of age on PD. The inpatient controls were included (Becker et al. 1997). According to a recent NAPRTCS analysis by Carey et al., the hospitalization times were longer in neonates compared to children between one month and two years at dialysis initiation (54 vs. 39 days,  $p < 0.001$ ). Moreover, some children were not hospitalized at all, which raises the question of whether the children had only outpatient controls



or if the ward controls were excluded from the data. Most of the patients were on PD (<10% on HD) (Carey et al. 2007). Besides, the authors do not report whether the days are total per dialysis time or per patient-year, and thus comparison to our data is not possible. There are some reports about hospitalization in children on HD, in children with CKD or ESRF but the exact hospitalization days or the PD patients are not separately reported. The number of hospitalization days in our study seems to be higher compared to other reports. We, however, include the training of the family, controls, and pretransplantation studies, in addition to the treatment of complications, to the hospitalization days, which probably raises the total number significantly. Our protocol controls occurred at one and three months after PD onset and every third month thereafter. This was to ensure proper nutrition, metabolic control, PD adequacy evaluations, and good growth to our patients. Almost all of our patients come from far away to our center and thus, outpatient controls are not useful in time-consuming studies such as adequacy measurements.

## **6.5 Growth in children on peritoneal dialysis (I, II)**

Most of our patients grew well during PD. Catch-up growth was seen in 64% of patients between 1995 and 2000 and in 57% during 2001–2005. In the retrospectively analyzed patients, the mean hSDS at the beginning of PD was -1.5 and at the end of PD -1.2. The overall mean hSDS at the beginning of PD was -1.3 and at the end of PD -0.9 in both groups, when the patient with severe congenital heart disease was excluded from the retrospective group. In the prospectively studied group, the difference in hSDSs between PD end and start was statistically significant. When compared to other studies, these children were not small. Cansick et al. (Cansick et al. 2007) reported a mean hSDS of -2.06 in children with a mean age of 2.8 years at PD onset. In children under two years of age, they noticed a mean change of +0.31 in the hSDS during the first year on dialysis. They found no correlation between hSDS change and alphacalcidol or phosphate binder doses, mean hemoglobin, sodium, phosphate, albumin, or PTH levels, as was also the case in our patients. In the study by Cansick et al., the median PTH was 1.5 times the upper limit of normal (ULN), while in our patients it was about twice the ULN after three months and about thrice the ULN after six months on PD. However, Cansick et al. do not reveal the time point of their PTH measurements, or the dosage of alphacalcidol, and thus a direct comparison to our data is impossible. We tried to improve growth with daily administration of alphacalcidol. However, control of iPTH was difficult, and pulse therapy was needed for many patients. Furthermore, Saarinen et al. (2007) showed, in a small PD patient population, that growth was better with pulse therapy of vitamin D. Schroff et al. (2006) reported a mean hSDS of -3.6 at the beginning of dialysis (PD or HD) and a final hSDS of -2.6 in children under five years of age. Those children were clearly smaller than our patients, with the youngest patients having the greatest growth deficit, however, the change in hSDS was not statistically significant. In the NAPRTCS report of 2007, the mean hSDS in

children commencing dialysis was -2.55 in children under two years of age, with PD patients being smaller than HD patients (NAPRTCS 2007 Annual Report 2008). The mean waiting time for a kidney transplant at our institution is only 9 months and as a consequence, our follow-up time for growth is relatively short. However, our patients clearly achieve normal growth during PD, with catch-up growth in 50% of patients, when appropriate medication and adequate nutrition are provided.

None of the earlier reports on children on PD have compared height with their midparental height. Our prospectively studied PD patients had a normal mean hSDS at birth (-0.69). Most of them grew normally for age during PD but they clearly lagged their midparental height at around 18 to 24 months of age. This is because they lost relative height prior to PD. In a very recent report by Tainio et al. (Tainio et al. in press), the authors report the final adult heights for 105 patients with renal Tx at a median age of 4.5 years. In males the mean final hSDS was -1.2 (168.7 cm) and in females -1.7 (153.7 cm). Their growth duration was prolonged and final heights were reached at a mean age of 18.1 in males and 16.0 years in females. Both genders with kidney Tx before the age of five years reached puberty earlier compared to older transplant patients, the mean difference being around one year. Thirteen per cent of the patients received rhGH therapy before and 30% after the renal Tx, while 56% did not get rhGH therapy at any time (Tainio et al. in press). The mean hSDS in boys is comparable to our growth measures in small children at PD end. However, the mean final hSDS in girls is clearly lower than in our prospective group at the end of PD. The patients in the Tainio et al. study were treated earlier than our patients and young patients on PD or with a kidney Tx might grow better today. After Tx there are also other confusing factors affecting growth such as corticosteroids as a part of immunosuppressive medication, and a direct comparison of these patient groups is not possible.

We did not have GH in our study protocol and thus we refrained from GH treatment in this age group. In our opinion, nutrition and proper management of ESRF (dialysis, medication) must first be instituted, and thereafter GH treatment can be considered if the child still is growing poorly.

## **6.6 Neurological development in patients treated with PD (III)**

In 48% of the prospectively followed PD patients, no risk factors were detected for impaired neurological development before PD initiation; 30% of the patients had normal neurodevelopment. Only minor impairments such as specific language impairment or attention deficit disorders were seen, altogether in 70% of these patients. However, more than half of the patients in the prospective group (11 of 21, 52%) had some predialysis risk factor for their development. The risk factors included pre-, peri- and neonatal risk factors and co-morbidities. Three of the patients with risk factors (27%), had normal neurodevelopment. Eight of the eleven risk factor patients (73%), had neurological impairment at the end of this study. Six of those eight (75%), had a major impairment. On the other hand, six of all these twenty-one prospectively

followed PD patients had some co-morbidity or syndrome in addition to their renal disease. The one with severe hypoglycemia as a newborn, and severe hypotension at two weeks, had normal neurodevelopment. All other patients with co-morbidity (five of six, altogether 83%) developed a neurological impairment during the study period and for most of them it was a major one. The prevalence of any neurological impairment was similar in both groups: in patients with or without predialysis risk factors, but only the patients with risk factors had major impairments. The proportion of patients with a neurological abnormality was higher than we expected. Yet, we report very mild impairments, such as dyspraxia and other problems, which may disappear within time, and presumably these minor problems do not cause permanent impairment. Very few studies report a neurological outcome in young PD patients. Warady et al. (1999) reported a small group of patients (n=28) treated with PD in infancy. They excluded all children with co-morbidities and neurological diseases from their study. Thus, the comparison between their study and ours is impossible. At the age of one year, they saw a neurological delay in 21% of the patients. Half of their patients were, however, hypotonic at one year. A considerable proportion of patients were also tested with an assessment of global intelligence from the age of 4 years and in 79% of the patients the performance was in an average range. One patient was deaf, but the authors did not report his/her performance separately. Honda et al. (1995) reported abnormal development in 69% of 15 patients at the end of CAPD. Their PD treatment had been started before 2 years of age. Our aim was to analyze the neurological development of all children dialyzed in infancy or before two years of age. Thus, we also included patients with co-morbid conditions and clear neonatal risk factors in our study.

Prospectively followed patients underwent no notable periods of additional diseases or severe hypo- or hypertensive crises during PD. The patient with NPHS1 and intrauterine alcohol exposure had one longer period with hypertension during PD. He developed attention deficit disorder and visuomotor disability, which are not typical consequences of hypertension problems. PD did not worsen the neurological status of the prospectively studied patients who were followed with examinations by a pediatric neurologist and AIMS tests.

CP was diagnosed in 14% of the prospectively followed PD patients, all of them displaying explanatory findings in the brain MRI. The prevalence of CP in our patients is similar to that seen in extremely-low-birth-weight infants (11%) in Finland (Tommiska et al. 2003), and clearly higher than in the whole population (2 to 3 newborns in 1,000) (The Finnish CP Association 2010). All our patients with CP had premature birth but none of them had extremely low birth weight.

Even after the neurological follow-up study on the prospective PD patients, it is difficult to define the etiology of neurological abnormalities in every case. Most patients had a clear reason for the impairment, such as brain infarct or co-morbidity. With the methods of assessments used here, we saw no worsening of the neurological condition during PD. There were seven patients without predialysis risk factors who developed a minor impairment. These impairments were not detected during PD but later with neuropsychological tests, at approximately five years of age. Only one of

them had changes in her brain MRI, detected during PD in pretransplantation studies. The etiology of this finding remains unclear. In six other patients with no risk factors, but with a minor impairment, we failed to find any sure explanation for their condition. We did not ask or analyze the existence of learning difficulties or other neurological aberrations in the parents. We were not able to analyze all minor predialysis factors such as degree and duration of acidosis or hypertension, adequacy of nutrition, or psychosocial factors in these children. On the grounds of our study, it is possible to say that the children with major impairment as well as two patients with minor impairment had clear reasons for their disability, but the etiology of their abnormality remained unsolved in most children with minor impairment.

## **6.7 NPHS1 with muscular dystonia and athetosis (IV)**

Most of the NPHS1 patients with muscular dystonia and athetosis (MDA) died at an early age. Those who survived are severely handicapped and need outside support to manage their everyday life. The reason for this newly described neurological condition in NPHS1 patients could not be clarified. If the reason lies in the chromosomes or in the mtDNA, we should see new patients with MDA. After our report on NPHS1 with MDA, one NPHS1 patient with neurological symptoms which might be MDA has been born but her diagnosis is not yet certain. All NPHS1 patients have significantly low plasma albumin values, especially before diagnosis and subsequent daily albumin infusions. Almost all of them are also preterm and thus prone to bilirubin toxicity. Hypoalbuminemia predisposes NPHS1 patients to bilirubin toxicity and chronic bilirubin encephalopathy, kernicterus, while albumin is an important bilirubin binding protein in the blood. Free unconjugated bilirubin affects the central nervous system and may cause bilateral lesions in the globus pallidus and subthalamus regions, seen in the brain MRI. It may also cause a hearing defect and abnormal brainstem auditory evoked potentials (Shapiro 2003). Kernicterus is still seen, but it is a very rare condition with an incidence of 1 per 40,000 to 150,000 in live births in the Western world. The children with bilirubin encephalopathy first have a tendency to opisthotonus, then probably seizures and apnea. Within time they usually develop an athetoid cerebral palsy, hearing loss, dental dysplasia, paralysis of upward gaze, and other handicaps (Maisels 2009). Many of these symptoms were also seen in our NPHS1+MDA patients. They also had increased signal intensity in the globus pallidus area in the brain MRI. Thus, there are plenty of facts supporting the possibility of kernicterus as a cause for the neurological syndrome in our patients. However, a statistical difference in the bilirubin levels as a newborn was not seen between the NPHS1+MDA patients compared to the controls.

In the future, it is important to document an exceptionally heavy placenta and edema and exclude NPHS1. In NPHS1 patients, intravenous albumin infusions should be started soon after detection of a low plasma albumin level and they should probably always be treated with phototherapy even in case of relatively low bilirubin levels to avoid possible neurological sequelae.

## 6.8 Limitations of the study

The mean duration of PD was only  $1.1 \pm 0.6$  years in our prospectively studied patients and  $1.4 \pm 0.8$  years in our retrospective patient group. In two thirds of the patients (n from 15 to 16) the dialysis period was at least nine months. This relatively short PD duration allows only a short follow-up time for growth. There are also many confounding factors affecting growth such as co-morbidities, nutrition, and infections.

Bone biopsies were not taken to aid evaluation of Ca-Pi metabolism, growth and bone health. Thus, we could not say anything about bone health itself but tried to follow-up and describe factors having an effect on bone growth, such as iPTH and Ca-Pi balance. Vitamin D levels were not measured in blood, which would have been interesting for evaluating bone metabolism and vitamin D substitution. Nevertheless, adynamic bone disease can be excluded as growth was normal. Alkaline phosphatase (AFOS) levels were also normal.

Problems were encountered regarding BIA measurements, due to the lack of a healthy control group. Thereby the BIA values gave very little information about the intravascular volume status of our patients.

Neuropsychological tests were not included in our study plan but they are a part of follow-up in all transplanted children. Study time points and investigators thus vary slightly. Also, the methods used vary between hospitals. Thus, the information from our neuropsychological tests is not totally uniform. They offer important information but do not provide critical information about these patients.

## 6.9 Future considerations

Evaluation of cognition is not yet possible, however in the future it would be interesting as well as important to arrange a follow-up study and evaluate these children at school age with congruent study assessments.

It would be interesting to further analyze the possibility of chronic bilirubin encephalopathy in some NPHS1 patients. No difference in bilirubin concentrations between MDA patients and normal CNF controls were observed in this study. It would be important to know possible factors affecting a bilirubin induced injury of the central nervous system, whilst all NPHS1 patients have low plasma albumin levels, why do only a small proportion of patients with high plasma bilirubin present neurological symptoms? Are some patients more vulnerable to injury or do some patients have protecting factors? Or is this neurological lesion caused by some other factor?

The decision to start or to refuse dialysis treatment in a small child, that is to start active treatment targeting at renal Tx, must include the discussion of the child's possible co-morbidity. If the co-morbidity is fatal, active treatment should not be initiated. In our center, every infant with ESRF has been actively treated if they have survived until PD is started. In children with a co-morbidity or neurological injury, the decision about active treatment is a difficult ethical problem. However, when we look back now, at

these children with neurological impairments, we would not have refused any of them active treatment.

It is important to evaluate the growth in these patients after renal Tx, to see if catch-up growth continues or starts later. The final height of these patients is of great interest. To be able to assure good growth in the future in these small children with ESRF, an early diagnosis of CKD as well as careful planning of the treatment and nutrition are important already before PD. Whether the predicted height could be achieved in that case, remains to be seen later.

PD treatment that is carried out at home, usually for many months, requires a lot of contribution from the family. In the future, it is important to evaluate the quality of life of the families, their resources, and how they cope with this exceptional situation. The parents have to be nurses in addition to parents. Furthermore, we are evaluating the parent-child relationship in families with a child with ESRF and peritoneal dialysis.

## 7 Conclusions

The studies I-IV evaluate dialysis outcome, growth, and neurological development in children less than two years of age at the onset of PD and in a group of NPHS1 patients with a neurological syndrome.

The main conclusions are the following:

1) Metabolic control was good in infants treated between 1995 and 2005. Reaching a balance in calcium-phosphorus metabolism was demanding and iPTH levels were difficult to control. Daily administration of vitamin D did not control the patients' secondary hyperparathyroidism and many patients were switched to pulse therapy to reach optimal iPTH levels. The reduction of dietary protein should be implemented after the first months of PD to reduce calcium based phosphate binders. Thus, the risk for vascular calcification can be diminished.

2) Hypertension was a common problem. Evaluation of intravascular volume status with existing tools is demanding. However, less antihypertensive medication was used between 2001 and 2005 than earlier. Fifty-seven per cent of patients treated between 2001 and 2005 had long-term hypertension, yet most of them without antihypertensive medication.

3) Growth during PD was good in the whole study population and catch-up growth was seen in most patients. Careful planning and implementation of nutrition and an adequate dialysis dose are important factors in assuring growth. Although the children grew well, they were significantly smaller at the end of PD than their predicted midparental height, calculated from the heights of their parents.

4) We found neurological impairment in 15 out of 21 prospectively studied patients at the end of the follow-up. In 29% of the cases, there was a major impairment and in 43% only some minor ones. Impairments were seen especially in patients with neonatal, perinatal or co-morbidity-related risk factors but milder ones in patients without risk-factors. All patients with major impairment had a predialysis risk factor for their development. PD did not worsen the neurological condition. Of these 21 prospectively studied patients, 19% had a brain infarct and 14% some other ischemic lesion or PVL. However, all school-aged children attended full-time school. Part-time special education was needed in 13% and full-time in 33%.

5) NPHS1 with MDA is a new syndrome, not described earlier. We did not find any clear explanation for the symptoms of these patients although we analyzed their mitochondrial genome and evaluated risk factors for neurological injury. Hyperbilirubinemia together with hypoalbuminemia amplifies the toxicity of bilirubin and kernicterus is a possible explanation, however, this could not be proven within the scope of this study.

6) Based on the above findings we have focused more on early diagnosis and aggressive RRT from the beginning and have adjusted our follow-up program. More focus is given on Ca-Pi balance, blood pressure follow-up and therapy of hypertension. In infants and small children, lower Crcl levels; over 40 L/week per 1.73 m<sup>2</sup> can be allowed even in anuric patients if they grow well. In addition to regular follow-up of blood pressure, left ventricular mass and the presence of LVH (with the method of Foster et al.) should be evaluated regularly during PD. Children with risk factors for developmental impairment are carefully supervised to characterize their problems and to minimize their developmental sequelae.



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